Journal of Food Protection, Vol. 72, No. 5, 2009, Pages 1121–1138

Review

Stress, Sublethal Injury, Resuscitation, and Virulence of Bacterial Foodborne Pathogens†

ALISSA M. WESCHE,[1] JOSHUA B. GURTLER,[2] BRADLEY P. MARKS,[3] AND ELLIOT T. RYSER[1]*

[1]*Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824;* [2]*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038; and* [3]*Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, Michigan 48824, USA*

MS 08-480: Received 25 September 2008/Accepted 20 December 2008

ABSTRACT

Environmental stress and food preservation methods (e.g., heating, chilling, acidity, and alkalinity) are known to induce adaptive responses within the bacterial cell. Microorganisms that survive a given stress often gain resistance to that stress or other stresses via cross-protection. The physiological state of a bacterium is an important consideration when studying its response to food preservation techniques. This article reviews the various definitions of injury and stress, sublethal injury of bacteria, stresses that cause this injury, stress adaptation, cellular repair and response mechanisms, the role of reactive oxygen species in bacterial injury and resuscitation, and the potential for cross-protection and enhanced virulence as a result of various stress conditions.

DEFINITION OF INJURY

Bacterial injury may be defined simply as the effect of one or more sublethal treatment on a microorganism *(74)*. By extension, sublethal injury is a consequence of exposure to a chemical or physical process that damages but does not kill a microorganism *(73, 175)*. Yousef and Courtney *(218)* include damage to cellular components in their description of injury, and Gilbert *(57)* wrote, "Sublethal injury of microorganisms implies damage to structures within the cells, the expression of which entails some loss of cell function that may be transient or permanent." Most intervention strategies used for the control of pathogenic and spoilage microorganisms frequently produce a continuum of sublethal effects, and a considerable proportion of microorganisms in foods likely incur some degree of sublethal injury during food processing *(80, 138, 222)*.

DEFINITION OF STRESS

The term *stress* has been used to describe the effect of sublethal treatments. However, Hurst *(74)* considers *injury* to be the preferred term because, by analogy with higher organisms, its description evokes an image of "temporary and repairable physical damage." By similar analogy, the term *stress* carries a more subtle meaning, not necessarily causing physical damage but altering organism behavior. Current literature pertaining to microbial injury typically does not maintain this distinction, and the terms are often used interchangeably. The term *stress,* however, is universally used in reference to the agents or treatments causing injury. Although there is a tendency to perceive food matrices as metabolically supportive environments, food is frequently bacteriostatic or bactericidal due to intrinsic factors such as water activity (a_w), pH, oxidation-reduction potential, competitive exclusion by protective cultures, and other environmental and processing stresses *(8)* (Tables 1 and 2). Other types of stress encountered in food environments may include exposure to acids, bases, bioactive antimicrobial peptides, oxidants, osmotic pressure differences, starvation, heating, freezing, thawing, and the presence of other innate and supplemented antimicrobial compounds *(136)*. Some emerging technologies (e.g., high hydrostatic pressure) cause sublethal injury, although some have argued that other technologies (e.g., pulsed electric field) do not induce injury *(215, 218)*. Bacterial stresses, which generally fit into three categories—physical, chemical, or nutritional—can occur throughout the farm-to-fork continuum and lead to different types of bacterial cell damage.

Storz and Hengge-Aronis *(193)* describe stress as any departure from optimal conditions with the potential to decrease bacterial growth. Situations that induce the expression of genes that respond to specific environmental conditions may also define a stressful situation and subsequently prove important in inducing bacterial stress responses during host-pathogen interactions.

Given that the perception of bacterial stress is coordinately linked to characteristics of the individual cell, merging the previous definitions of stress into one universal definition is difficult. A working definition of bacterial stress from a food microbiology perspective is typically taken to be a physical, chemical, or nutritional condition insufficiently severe to kill, resulting in sublethally injured mi-

* Author for correspondence. Tel: 517-355-8474, Ext 185; Fax: 517-353-8963; E-mail: ryser@msu.edu.

† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

TABLE 1. *Physical and chemical treatments (stresses) able to reversibly injure microorganisms*

Treatment	Reference(s)
Physical	
Drying, including air, vacuum, and freeze-drying	*167, 168*
Heat, particularly sublethal heating processes	*167, 168, 215*
High hydrostatic pressure	*168, 215*
Low temperatures, including both refrigeration and freezing	*167, 168*
Pulsed white light	*215*
Radiation: gamma, UV, and X ray	*167, 168*
Solids: concentrations of sugars, salts	*167, 168*
Chemical	
Chemical sanitizers: chlorine, iodine, quaternary ammonium compounds	*167, 168*
Oxidative treatments, including ozone, H_2O_2, lactoperoxidase system	*197*
pH: alkali and acids (organic and inorganic)	*167, 168*
Preservatives: sorbate, benzoate, nitrate, bacteriocins, etc.	*167, 168*

TABLE 2. *Microorganisms known to experience reversible injury from sublethal stresses important in food systems*

Microorganism	Primary importance in food	Reference(s)
Escherichia coli	Indicator, pathogenic	*167, 168*
Enterobacter aerogenes	Indicator	*167, 168*
E. sakazakii	Pathogenic	*66*
Klebsiella spp.	Indicator, pathogenic	*167, 168*
Enterococcus faecalis	Indicator	*167, 168*
Salmonella	Pathogenic	*167, 168*
Listeria monocytogenes	Pathogenic	*168, 217*
Shigella	Pathogenic	*167, 168*
Vibrio parahaemolyticus	Pathogenic	*167, 168*
Yersinia enterocolitica	Pathogenic	*167, 168*
Campylobacter jejuni	Pathogenic	*167, 168*
Staphylococcus aureus	Pathogenic	*167, 168*
Clostridium perfringens	Pathogenic	*167, 168*
C. botulinum	Pathogenic	*168*
Pseudomonas spp.	Spoilage	*167, 168*
Bacillus spp.	Spoilage, pathogenic	*167, 168*
Lactococcus lactis	Bioprocessing (fermentation)	*168*
Lactobacillus bulgaricus	Bioprocessing (fermentation)	
L. acidophilus	Dietary adjunct (probiotic)	
Saccharomyces cerevisiae	Bioprocessing (fermentation)	*167*
Candida spp.	Spoilage	*167*
Aspergillus flavus	Spoilage	*167*

crobes *(73, 142)*. These conditions can be processing treatments, but they may also include environmental conditions, such as limited nutrients in water.

DEGREES OF BACTERIAL INJURY, CELLULAR ADAPTATION, AND RESUSCITATION

It has also been postulated that there are different levels of stress, which progress in severity from minor to moderate, severe, extreme, and eventually lethal *(193)*. With minor stress, bacterial cells adapt completely to the changed conditions, and growth rate is not affected. Low levels of stress may cause a transient adaptation (adaptive response) accompanied by a temporary physiological change that often results in increased stress tolerance *(218)*. Lethal stress, however, can cause the death of some, but not necessarily all, bacterial cells. When lethality is experienced by only a fraction of the population, accompanying gene responses and adaptive mutations may actually improve survival of the overall population *(8, 193)*. Moderate stress may result in a continuum of injury, ranging from mild to severe, including healthy and dead cells *(74, 75, 114, 191)*. The relationship between the different levels of stress and degrees of injury and the ability of cells to adapt under these conditions is not well defined. For all practical purposes, *sublethal injury* includes any injury short of death with the organism frequently undergoing some type of stress adaptation.

The expression of sublethal injury can be manifested in several ways in the laboratory *(73)*. *Metabolic injury* has been described as the inability of bacterial cells to grow on defined minimal media *(57)*. This inability to multiply is often temporary, after which resuscitation will occur *(74)*. *Structural injury* is viewed as the inability of bacteria to proliferate or survive in media containing selective agents (e.g., bile salts, tellurite, sodium desoxycholate, bismuth sulfite, oxgall, sodium azide, sodium chloride, crystal violet, brilliant green, selective antibiotics, tergitol, or other surfactants), with no obvious inhibitory effects on unstressed cells *(57, 73, 74, 168, 179)*. Depending on other conditions (e.g., enrichment, incubation conditions), the inability of cells to form colonies on selective media may also be temporary, which would once again indicate that metabolic repair from injury can occur if provided sufficient recovery time under appropriate environmental conditions.

Some authors consider metabolic and structural injury to be varying degrees of the same type of injury *(74, 88)*. Metabolic and structural injury are manifested by the loss of characteristic growth capabilities and the inability to form visible colonies under selective conditions under which uninjured cells are able to form colonies *(27, 73)*. However, it seems that while injured cells typically suffer structural damage, such as might affect cell wall or membrane permeability, metabolic injury includes damage more extensive to various functional components of the cell *(23)*. Consequently, the growth or no growth response on selective media is the traditional means of assessing sublethal injury *(23, 33, 57, 74, 179)*. The difference in plate counts between selective and nonselective media is used to quantify sublethal injury as a proportion or percentage of the entire population *(15, 23, 39, 88, 170, 179)*.

Incubation temperature has also proven crucial in cellular repair, resuscitation, and colony development by foodborne pathogens. Lucht et al. *(111)* reported that incubating

TABLE 3. *Optimal resuscitation temperatures for recovering gamma-irradiated–injured bacteria (adapted from Lucht et al. (111))*

Bacterium	Radiation exposure (kGy)	Optimal recovery temp (°C)
Escherichia coli 11775	1.6	15–22
E. coli O157:H7	0.2–0.6	5–18
Salmonella Typhimurium	0.5–1.5	22
Yersinia enterocolitica	0.6	14
Listeria monocytogenes Scott A	0.32–0.85	5–37
L. monocytogenes 81–861	0.6	37
L. innocua	0.55	37
L. ivanovii	0.55	37
Pseudomonas fluorescens	0.15	22
Staphylococcus aureus	0.6	2
Bacillus subtilis	0.48	18
Aeromonas hydrophila	0.18	22
Brochothrix thermosphacta	0.48	18

radiation-treated bacteria at suboptimal temperatures was more effective in recovering injured cells than was incubating at the optimal growth temperature (Table 3). This is similar to earlier findings regarding the resuscitation of irradiated- and heat-injured bacteria *(1, 159, 188).* Incubation of injured bacteria at suboptimal growth temperatures reportedly suppresses cell division while permitting metabolic repair processes to continue, presumably reversing the effects of sublethal injury prior to replication. Bacterial repair and resuscitation are dealt with more extensively in a latter section of this article.

SOURCES OF BACTERIAL STRESS

Acid and alkaline stress. Acute acid shock or gradual acid stress can occur in low pH conditions when H^+ ions cross the bacterial cell membrane and create an acidic intracellular pH. Likewise, undissociated organic acids can diffuse across the bacterial cell membrane and lower the internal pH on dissociation *(2, 51).* While acid injury is thought to be the result of an easily alterable pH equilibrium shift, reversing the process is more complex than is simple neutralization of the acidic conditions *(160).*

Acid stress can occur during the fermentation of foods or by the addition of preservatives such as organic acids *(2, 184).* Current recommendations for the use of acid washes to decontaminate beef carcasses include 1 to 2.5% lactic acid *(56).* Dickson and Siragusa *(40)* found that washing beef tissue with 1% lactic or acetic acid sublethally injured *Salmonella* Typhimurium on carcasses.

Likewise, alkaline stress can occur under increased pH conditions. Many detergents and chemical sanitizers, such as caustic soda (NaOH) and ammonium compounds, are used to clean food processing facilities and food contact surfaces *(196).*

Starvation stress. Starvation stress can occur on animal carcasses, in food, on equipment surfaces, walls, floors, and in water *(39, 109).* Dickson and Frank *(39)* defined *starvation stress* as the survival of bacteria in oligotrophic environments, which are environs with adequate oxygen concentrations but low or no available nutrients to support biochemical metabolic activities and multiplication. Natural environments typically have growth-limiting levels of nutrients and rapidly changing nutrient availability *(59, 69).* Bacteria are present in different physiological states during the lag phase, exponential growth, slow growth, the stationary phase, and death. Cryptic growth, the phenomenon that occurs when dead organisms provide nutrients for the multiplication of survivors *(158),* may also contribute to the survival of populations well after growth has ceased.

Cold stress. *Salmonella* can reportedly survive during cold storage at 5°C for up to 8 months *(36, 81).* This is likely due to cold shock and subsequent adaptation. The cold shock phenomenon occurs when growing bacteria are exposed to a sudden temperature drop of at least 10°C, leading to cold shock in susceptible microorganisms *(84, 113).* The associated cold shock response is divided into stages of initial cessation of growth, resumption of growth after an adaptive period, and changes in protein synthesis *(136).* Microorganisms inhabiting foods that must be refrigerated for pre- and/or postprocessing storage are subject to cold shock. Additionally, injury due to cold shock may occur if serial dilutions of microorganisms are held in the refrigerator when laboratory tests cannot be immediately completed *(205).*

Sensitivity of bacteria to low temperatures varies widely and is based on population density, growth temperature, cooling rate, and the temperature range over which cooling occurs *(113, 158).* The effect of food components on the degree of injury and survival of cold-stressed microorganisms has not been studied in great detail. Preliminary work has shown that water or low-nutrient diluents present more stressful environments than do nutrient-rich broths or food *(113).* The time required to reduce *Salmonella* by 90% in water at 0 to 5°C was between 2 and 16 days, with populations in vacuum-packaged beef decreasing about 50% in the same temperature range after 28 days of storage *(95, 113, 137).* However, *Vibrio vulnificus* decreased nearly 7 log in oyster homogenate after 24 h of storage at 4°C, due to lethal cold stress, with no reduction seen in a salt-based culture medium during the same time period *(147).*

Freeze injury. Although injurious to bacteria, freezing is generally recognized as an ineffective microbial inactivation strategy. Freeze injury results from continued exposure to concentrated solutes and physical damage caused by ice crystal formation. Many constituents of food and culture media are protective against freeze damage. These cryoprotectants include glycerol, sodium glutamate, certain sugars, peptides, and proteins *(113).* Utilization of cryoprotectants in one study may have contributed to minimizing the impact of freeze injury on *Escherichia coli* O157:H7 *(179).*

Osmotic stress. Osmotic stress that is associated with freezing also occurs during freeze-drying, when microorganisms are subjected to freezing, drying, storage, and rehydration stresses *(113).* Rapid rehydration of foods and freeze-dried cultures can cause osmotic injury *(205).* Os-

TABLE 4. *Sites of cellular injury after exposure to various forms of sublethal treatments (adapted from (138, 166, 173, 215))*

Sublethal treatment	Cell wall or cell wall synthesis	Membrane (cell leakage)	Proteins	RNA (ribosomes) or RNA synthesis	DNA or DNA synthesis
Freezing		√		√	√
Drying	√	√		√	√
Freeze-drying	√	√		√	√
Heating		√	√	√	√
Gamma radiation	√	√	√	?	√
Change in oxygen potential		√			
Osmotic shock	√	√			
Starvation			?	√	
High hydrostatic pressure		√	√		
Pulsed white light		√	√		√
Salt shock			√		

motic stress can occur when shifts in external osmolarity cause water to flow either into or out of the bacterial cell and in extreme conditions leads to physical damage of the cell. Less severe changes in osmolarity manifested by the presence of NaCl or other salts or solutes affects the availability of free water to the cell *(24)*. Water availability is intimately linked to a_w. In food processing, microorganisms respond to both "storage a_w" and "treatment a_w" *(162)*.

Heat shock. In contrast to other bacterial stresses, heat shock is perhaps the most studied and best understood. Heat shock occurs when organisms are exposed to temperatures above their normal growth range *(26, 47, 116, 148)*. Temperatures at which heat injury is induced may be lethal to a fraction of the bacterial population, based on the growth phase and heat sensitivity of the microorganism *(142)*.

When processing animal carcasses, hot acid sprays may elevate the superficial temperature of the carcass, altering the growth and resistance profile of indigenous flora *(30)*. Conditions within both preprocessing and processing environs may cause heat shock or stimulate a heat shock response in the target microorganism. Guidelines for the use of acid washes to decontaminate animal carcasses include spray temperatures that vary from 20 to 60°C *(56)*. The heat shock response has been reported to occur at a temperature as low as 42°C for *E. coli* O157:H7 *(142)*, 46°C for *Campylobacter jejuni (150)*, 48°C for *Salmonella* Typhimurium *(26, 116)*, and 45 to 48°C for *Listeria monocytogenes* within a fermented beef–pork sausage homogenate *(47)*.

Thermal processes that include extended come-up phases, such as low-temperature pasteurization of eggs, slow roasting of certain meat products, or certain sous-vide processes, might generate conditions that can induce sublethal thermal injury to microorganisms *(26, 118, 142, 148)*. The behavior of microorganisms in foods that are heated gradually may mimic the response of microorganisms to isothermal heat shock, resulting in a concomitant genetic and physiological heat shock response *(47, 117, 163)*. Likewise, microorganisms present in meat products left on warming trays before receiving a final reheating could also experience heat shock *(47)*.

STRESS-INDUCED CELLULAR MODIFICATION AND INJURY

Bacterial stresses induced via environmental and processing conditions may result in decreased structural cell function and/or protein denaturation *(109)*. Table 4 outlines those cellular sites damaged by various sublethal treatments.

Structural modification. Dead, injured, or otherwise-robust cells likely differ in the degree of injury that may be imposed on structural and functional components *(165)*. Morphological changes in the cellular shape of *Salmonella, E. coli, Listeria, Pseudomonas, Aeromonas,* and *Lactobacillus* have been documented after sublethal injury *(126, 127, 133)*. McMahon et al. *(133)* found that exponential-phase *Salmonella* Virchow cells osmotically stressed in a 5% NaCl solution for 24 h were 54% filamentous and 30% spherical, versus stationary-phase stressed populations that were 16% filamentous and 79% spherical.

pH stress. Exposure to low-pH environs may remove or sequester cations from key metabolic sites within a microorganism *(61)*. Damage to RNA has been associated with low pH, and could be due to removal of magnesium (Mg^{2+}) *(74, 160)*, which is integral to ribosomal integrity. Acetic acid has been reported to interfere with the ribosome and subsequent protein synthesis *(74, 138)*. Shifts in pH can also disrupt the proton motive force *(173, 175)* and alter the protein profile of the outer membrane in gram-negative cells, the cytoplasmic membrane in gram-positive cells, or the protein coat of spores *(61, 104)*. Low cytoplasmic pH is also damaging to DNA *(173, 218)*.

Starvation stress. Nutrient deprivation can lead to an increase or decrease in bacterial exopolysaccharide levels *(39)* and effect entry into the stationary phase of growth, which is a period characterized by drastic changes in the cell envelope, membrane composition, and DNA structure, often due to the upregulation of global regulatory responses *(69, 173)*. Starvation actuates other physiological changes, including decreased cell size, decreased membrane fluidity, and increased protein turnover *(109)*. Microorganisms in nutritionally deficient environments likely integrate cell

density and starvation stress signals to effect cell surface modifications and utilization of alternate energy sources *(99, 158)*. Transformations in cellular morphology and cell surface components also enhance bacterial adherence and may contribute to biofilm formation.

Membrane damage. Damage to and modification of the cell membrane are associated with almost all forms of physical stress *(114)*, including injury by chilling, freezing, or heating *(22)*. Damage to the outer membrane of gram-negative cells leads to the release of lipopolysaccharides, lipids, phospholipids, divalent cations necessary for lipopolysaccharide stability, and periplasmic enzymes that may disrupt membrane permeability *(22, 73, 168)*. Permeability control is also lost at low temperatures because of decreased membrane fluidity *(63, 113)*, and alterations in permeability have also been associated with chlorination *(206)* and alkalinity *(177)*. Changes in permeability have led to the suggestion that certain treatments, such as heating, freezing, drying, and radiation, create small pores in the outer membrane *(114)*. However, resuscitative repair of stress-induced pores in the outer membrane does not necessarily facilitate repair of the cytoplasmic membrane *(73)*.

Protein and enzyme damage. While gram-positive organisms do not have an outer membrane, they do have a surface protein layer outside the cell wall, both of which are susceptible to injury. For example, freeze injury in *Lactobacillus bulgaricus* has been shown to damage the protein layer *(114, 214)*, and Mg^{2+} and D-alanine were lost from cell wall teichoic acid polymers of *Staphylococcus aureus* when heated in phosphate buffer *(75)*.

Certain treatments, particularly heating, lead to the inactivation of enzymes and disruption of the active transport of cations, sugars, and amino acids *(73)*. Dehydrogenases are especially heat sensitive. Glycosylases and endonucleases, enzymes involved in DNA repair, are heat labile at mild temperatures of 40 to 50°C *(153)*. When sublethally heat injured, *S. aureus* has reduced catabolic capabilities, and *Salmonella* Typhimurium exhibits a disruption in glucose transport *(27)*. Thermal treatment also can inactivate catalase and superoxide dismutase in several microorganisms, including *L. monocytogenes* and *S. aureus*, leading to an increased susceptibility to H_2O_2 and superoxide, and subsequent generation of the hydroxyl radical *(7, 34)*.

Ribosomal and RNA damage. VanBogelen and Neidhardt *(204)* theorized that ribosomes might function as sensors of conditions or temperatures to elicit a heat or cold shock response. Ribosome and ribosomal RNA (rRNA) degradation are often manifested as heat-induced lesions *(55, 198)*. In thermally stressed cells of *S. aureus, Salmonella* Typhimurium, and *E. coli*, the 30S ribosomal subunit is damaged or destroyed during destruction of 16S rRNA *(73)*. However, destruction of the 30S subunit can be prevented by supplementing the heating menstruum with Mg^{2+} as $MgCl_2$. Loss of ribosomes also occurs during starvation, particularly when conditions lead to intracellular Mg^{2+} depletion *(114)*. Since Mg^{2+} is necessary for ribosomal integrity and inhibition of ribonuclease, ribosomal damage largely appears to be a consequence of Mg^{2+} loss *(73)*.

DNA damage. While DNA damage has been observed in heated, freeze-thawed, dried, and acid-treated cells, it may be an indirect result of these treatments *(114, 175)*. For instance, DNA strand breakage during or after heat treatment is likely due to the stimulation of endonuclease activity *(73, 175)*. Death in cold-shocked *E. coli* is associated with the loss of Mg^{2+}, which is required by DNA ligase for DNA synthesis and repair *(178)*.

Effect of stress on lag time. Stephens et al. *(191)* assessed the growth of heat-injured *Salmonella* Typhimurium in microtiter plates by monitoring turbidity, and then calculated the lag times by using a model that extrapolated the growth curve back to the initial inoculum level. While the lag times for individual healthy cells were narrowly distributed, the lag times for injured cells ranged from <12 to >20 h, with some lag times >30 h. Variable lag times were also reported when *L. monocytogenes* was exposed to nine stresses, including acid or alkaline conditions, cold, heat, chlorine, and starvation *(65)*. The type of stress applied to *L. monocytogenes* affected the lag-time distribution, with lag-time variability attributed to heterogeneity among individual cells. One hypothesis for lag time variability is that lag time is determined by (i) the amount of work a cell must do to return to a condition that allows replication and (ii) the rate at which this work can be conducted *(65, 171)*. The heterogeneous nature of bacterial populations emphasizes the need for traditional and rapid methodologies to include preenrichment and repair steps, particularly when competing microflora may preclude recovery of injured cells.

STRESS RESPONSES AND REPAIR MECHANISMS

Bacterial response to thermal injury. Induction of certain stress-inducible proteins is coincident with the development of acquired resistance to heat (acquired thermotolerance) *(116, 144)* and other stresses (cross-protection) *(13, 109, 208)*, and can therefore not be used to establish a direct cause-and-effect relationship *(82, 87)*. However, heat shock and general stress responses may induce changes that protect cells from the effects of heat and other stress conditions *(80)*. The phenomenon of one type of stress-response imparting auxiliary protection to cells subsequently stressed at higher levels (especially for heat) is widely documented and may be referred to as "cross-protection." Lou and Yousef *(109, 110)* describe this as "stress hardening" and suggested that a bacterium previously exposed to sublethal stress is more likely to become adapted or hardened on exposure to subsequent stresses.

Watson *(208)* reported that acquired thermotolerance appears to be the most characteristic physiological response microorganisms have to mild temperature shock. Novak et al. *(145)* reported that vegetative cells of *Clostridium perfringens* incubated at 46°C for 60 min had significantly higher $D_{60°C}$-values than those cells that were incubated at 28°C. Exponential-phase cells of *Vibrio parahaemolyticus* heat shocked at 42°C for 30 min had a $D_{47°C}$-value of 3.33

min and were significantly more heat resistant than were non–heat-shocked cells having a $D_{47°C}$-value of 2.03 min *(211)*. Heat shocking *E. coli* O157:H7 at 42°C for 5 min before thermal inactivation at 55°C also increased the *D*-value more than twofold *(141, 142)*. Juneja et al. *(86)* sublethally heated samples of beef gravy and ground beef previously inoculated with a four-strain cocktail of *E. coli* O157:H7 at 46°C for 15 to 30 min. The heat-shocked cells were more thermotolerant than were unshocked cells, with a 1.56- and 1.50-fold increase in the time to achieve a 10,000-fold reduction at 60°C in beef gravy and ground beef, respectively. Similarly, after heat shock (30 min at 54°C) in comminuted turkey, an eight-strain cocktail of *Salmonella* exhibited an increased $D_{60°C}$-value (0.64 min) when compared with an unshocked control (0.41 min) *(210)*. When treated at 62°C, cells of *L. monocytogenes* that had been heat shocked in Trypticase soy broth (TSB) containing 0.6% yeast extract (TSB-YE) at 45°C for 180 min were sixfold more heat resistant than non–heat-shocked cells were *(149)*, and a heat shock in TSB-YE at 48°C for 10 min increased the $D_{55°C}$-value of *L. monocytogenes* more than twofold *(106)*. Mackey and Derrick *(118)* determined that *Salmonella* Thompson inoculated in TSB, liquid whole egg, and 10 or 40% reconstituted dried milk was more thermal tolerant at 54 or 60°C if held at 48°C for 30 min before treatment. The degree of thermal resistance and onset time has been shown to increase commensurate to increasing heat shock temperatures in *Salmonella* Typhimurium *(116)*.

The stress response of microorganisms in foods that have been heated slowly is thought to be the same as the response to an instantaneous heat shock. Evidence suggests that the rate at which a product is heated should be included when determining inactivation kinetics. Quintavalla and Campanini *(163)* found that *L. monocytogenes* was more heat resistant at 60, 63, and 66°C when heated slowly (0.5°C/min) in a pork emulsion than if heated virtually instantaneously at these temperatures. Mackey and Derrick *(117)* examined the heat resistance of *Salmonella* Typhimurium as affected by rising temperatures and concluded that slowly increasing the temperature (2, 1, and 0.6°C/min) from 20 up to 55°C, versus instantaneous heating at 55°C, increased thermal resistance. The resistance of cells increased as the heating rate decreased, with the greatest resistance afforded by a heat shock at 48°C for 30 min prior to inactivation heating. Stephens et al. *(189)* reported a similar increase in thermal resistance correlating to a reduction in heating rate for *L. monocytogenes*.

Entering the viable but nonculturable state as a means of protection. Any bacterial population in a processed food matrix will contain a continuum of disparately stressed cells that vary in their expression of stress response factors and in their ability to undergo complete resuscitation. Severely injured, yet metabolically active, cells of foodborne pathogens such as *Salmonella, E. coli, Shigella,* and *Campylobacter* that cannot be resuscitated under routine laboratory conditions can enter a viable-but-nonculturable state and maintain their pathogenicity *(10, 154, 164, 192)*. Panutdaporn et al. *(151)* demonstrated that a resuscitation and promoting factor produced by the *rRpf* gene cloned from *Salmonella* Typhimurium LT2 could transform NaCl-injured *Salmonella* Oranienburg from the viable-but-nonculturable to the culturable state. Genes responsible for the expression of resuscitation and promoting factors have been identified in various bacterial genera and may assist in promoting the growth of *Mycobacterium* spp. after prolonged dormancy *(67, 90, 95, 139)*. Under appropriate intrinsic (e.g., nutrients, a_w) and extrinsic (e.g., temperature, relative humidity) conditions, many injured cells will repair and regain the characteristics of normal cells at any point from preharvest to consumption *(28, 166)*, and exhibit increased stress tolerance as a result of stress adaptation. Their repair process is often reflected in delayed germination of spores, a prolonged lag phase for vegetative organisms, and the inability to multiply until fully repaired *(28)*. After repair, resistance to selective agents and the ability to proliferate in their presence is regained *(23)*.

Assessment of repair. Repair times may be estimated by measuring the time of the extended lag phase or by the differential plating method, further explained below. Using nonselective media, the estimated lag phase is the time required for the bacterium to recover from injury and begin multiplication. The differential plating method uses a selective medium containing a substance inhibitory to injured microorganisms (e.g., due to membrane damage) and a nonselective medium with no obvious inhibition of unstressed cells. The nonselective or "reference" medium enumerates the entire population, and the selective medium only recovers the healthy fraction. With differential plating, repair time is the time necessary to regain resistance to the selective medium. The time needed for repair depends on the bacterial species and strain, the type and severity of injury, and the inhibitory nature of the selective medium *(114)*.

Measurements with differential plating media suggest that, under optimal conditions, microorganisms injured by exposure to acid, chilling, freezing, freeze-drying, gamma radiation, or mild heating can become fully repaired within 4 to 5 h, with severe heat injury sometimes requiring much longer repair times *(114)*. Repair of *C. jejuni* subjected to heat shock in potassium phosphate buffer at 46°C for 45 min was complete within 4 h of incubation at 37°C, although no repair occurred at 5°C *(150)*. Damage to *E. coli* caused by sublethal acidification (sodium acetate buffer, pH 4.2, 60 min) was completely repaired after 1 to 2 h at 32°C in TSB, with 95% repair reported after 120 min in potassium phosphate buffer (pH 8.0) *(160)*. Freeze- and alkali-injured *E. coli* cells have also been shown to recover when incubated in phosphate buffer *(143)*.

Repair times determined by estimating lag time do not always agree with those obtained from differential plating. Mackey and Derrick *(115)* showed that gamma-irradiated and desiccated *Salmonella* Typhimurium cells sometimes recovered full resistance to the selective medium before the lag phase was complete. In some instances, the required time for individual heat-injured cells of *Salmonella* Typhimurium to regain tolerance to salt (up to 14 h) was longer than the lag time (9 h). Mathew and Ryser *(124)* reported

growth of heat-injured *L. monocytogenes* within the first 2 h of incubation, although injury was not fully repaired as assessed by the differential plating method. Meyer and Donnelly *(135)* also reported growth of heat-injured *L. monocytogenes* before resistance to the selective medium was fully regained, suggesting that the differential plating method may provide a more accurate means to assess injured-cell recovery.

Lag and repair times for injured bacteria reported in the literature must consider cell-to-cell variability (including growth phase) during stress exposure. These differences lead to variations in injury within a population *(191)* and repair time, as determined by differential plating or estimation of lag phase, which can be biased due to bacterial heterogeneity. In general, less severely injured cells repair more quickly and have a shorter lag period than do more severely injured cells. When cells with a shorter lag period begin multiplying on nonselective and selective media, they can overshadow the presence of cells requiring longer periods for resuscitation. Consequently, a single or average repair time does not reflect the distribution within a population, due to varying degrees of injury and may well be an underestimation *(114, 115)*. This may help explain the growth observed by Mackey and Derrick *(115)*, Meyer and Donnelly *(135)*, and Mathew and Ryser *(124)*, before all cells regained resistance to selective agents.

Repair mechanisms. Repair requires specific biochemical events that will differ based on the type and degree of stress. Metabolic processes that occur during repair can include the synthesis of ATP, DNA, RNA, proteins, and the reorganization of existing macromolecules, including lipopolysaccharides in gram-negative bacteria and teichoic acid in gram-positive bacteria *(74, 167, 168)*. Bacteria also accumulate intracellular compounds that protect macromolecules and membranes from damage. In *E. coli*, trehalose has been recognized as the primary protective osmolyte, and trehalose biosynthesis is induced by osmotic shock, extreme heat and cold, desiccation, and entry into stationary phase *(161)*. Repair of the cell membrane through lipid synthesis must occur relatively rapidly so that cells can fully repair from stress-induced lesions *(20)*. Normal cellular functions may be reestablished by the transient synthesis of general or specific stress proteins *(109)*. Since heat shock proteins (HSPs) were first identified in *Drosophila* spp. in 1974, virtually every organism studied has been shown to respond to a moderate temperature shock by increased production of specific proteins *(26, 47, 116, 185, 208)*. Bacterial responses to stress can be general or specific. In some instances, a component of a given stress response may be part of both general and specific response pathways *(78)*.

Characteristics of stress-induced proteins include increased production under conditions that repress the synthesis of most other cellular proteins *(105)*, a functional role in adaptation of the cell to growth- and survival-limiting conditions *(207)*, and the overall facilitation of recovery from stress-induced damage *(26)*. Stress-induced proteins, including HSPs, in prokaryotes, eukaryotes, and archaebacteria are among the most evolutionarily conserved *(144, 185, 208)*.

Stress response proteins. The arbitrary classification of stress proteins into categories such as specific or general is often difficult because numerous factors may individually be capable of inducing their expression *(208)*. Many of the same stress proteins in *Bacillus subtilis, E. coli, Enterococcus faecalis,* and *Lactococcus lactis* are universally induced after exposure to different stresses *(58)*. Jenkins et al. *(82)* studied starvation that was followed by heat and hydrogen peroxide stress in *E. coli* and found that each stress produced its own distinct pattern of proteins. However, 11 heat shock and six oxidative stress proteins were common to starvation proteins, with three proteins common to all three stresses. Völker et al. *(207)* determined that the proteins induced by *B. subtilis* after heat stress were both general stress and heat shock–specific proteins, indicating that the heat shock and general stress responses are related. Some genes associated with the general stress response have clear functions for managing specific stresses, such as oxidative and osmotic stress *(218)*, while others play a role in general protection under multiple stress conditions *(78)*.

The general stress response mechanism. The general stress response in most gram-negative bacteria, including the enteric bacteria *E. coli, Shigella flexneri,* and *Salmonella* Typhimurium, is regulated by RpoS, the alternative sigma subunit of RNA polymerase *(2)*. In *E. coli*, RpoS controls the expression of more than 50 genes involved in the general stress response *(107)*. RpoS can be induced by several stresses, including nutrient starvation, osmotic shock, high and low temperatures, pH stress, and oxidative stress *(2, 70, 173)*. Dodd and Aldsworth *(42)* demonstrated induction of RpoS in *Salmonella* with changes in a_w produced by using various humectants and food preservatives. Bacteria defective in the gene for RpoS are more sensitive to different food processing conditions, including heat shock, starvation, acid, and ethanol exposure *(2, 169)*.

In *L. monocytogenes, S. aureus, B. subtilis,* and other gram-positive bacteria, the alternative sigma subunit σ^B regulates the general stress response, and σ^B controls the expression of over 40 genes in *B. subtilis (2)*. Like RpoS, σ^B is induced by exposure to various environmental stresses, including ethanol, nutrient starvation, oxygen limitation, low and high temperatures, high salt concentrations, and hydrogen peroxide *(2, 207)*. Disruption of σ^B in *B. subtilis* can increase sensitivity to oxidative stress, while its disruption in *L. monocytogenes* reportedly decreases resistance to acid and osmotic stress *(2)*.

Heat shock response. Like the general stress response, several specific stress responses are adaptive responses that allow bacteria to survive and, in some cases, multiply under stressful conditions *(2, 168)*. The heat shock response and associated HSPs are the responses most commonly reported in the literature. Several HSPs act as molecular chaperones or chaperonins. These chaperones help cells survive by refolding proteins or targeting for destruction those proteins that have been improperly assembled or denatured *(64, 86,*

105, 185). Some HSPs possess protease or peptidase activity for degrading irreparably denatured proteins *(86, 105, 185, 218).* HSPs may also play a role in DNA repair and replication, cell division, modification of cellular morphology (*E. coli* exhibits a transient tendency to elongate), and the accumulation of osmolytes that may maintain or enhance protein stability *(2, 146, 201).*

The heat shock response is theorized to be responsible for the synthesis of proteins that replace thermolabile components of macromolecule-synthesizing systems, such as ribosomes. Presence of an inducible rather than a constitutive heat shock system in bacteria suggests its involvement as a mediator during temperature shifts rather than during high temperatures *(144).* HSPs have been identified after several other stresses, including starvation and anaerobiosis, as well as exposure to ethanol, other organic solvents, oxidative agents, and high salt concentrations *(58, 82, 105, 173, 208).*

Accumulation of damaged or denatured proteins after environmental stress is also thought to trigger the synthesis of numerous proteins involved in repair, including HSPs *(208).* For example, numerous stress-inducible proteins and genes are known to be upregulated in the cell following thermal treatment. When Kobayashi et al. *(97)* thermally treated *Salmonella* Enteritidis at 55°C for 15 min in citric acid–disodium hydrogen phosphate buffer at pH 6.0, resuscitation for 1 h in TSB upregulated the transcription of 19 heat-inducible genes (*clpB, clpX, degP, dnaJ, fkpA, ftsJ, gapA, hflB, hslJ, hslU, hslV, htpG, htrA, lon, mopA, mopB, mreB, rpoE,* and *ppiD*) and 12 oxidative stress– and DNA damage–inducible genes (*ahpC, ahpF, fldB, fur, grxA, dinF, katG, mutM, recA, soxR, trxC,* and *zwf*), as well as two proteins, FusA (elongation factor G, EF-G) and PykF (pyruvate kinase), which increased in concentration by ca. eight- and sixfold, respectively. The propensity of stress-inducible mutations to occur in bacteria may work to promote the upregulation of survival proteins and genes. Highly pathogenic isolates of *E. coli* and *Salmonella enterica* are known to undergo mutagenesis at higher-than-normal frequencies compared with less virulent isolates, which might increase their ability to survive when exposed to less-than-optimal environmental and processing conditions *(100).*

Cold shock response. Many bacteria respond to abrupt decreases in temperature by transiently synthesizing a number of protective proteins *(63).* Although not studied in prokaryotes and eukaryotes to the same extent as HSPs, cold shock proteins (CSPs) have been shown in *Bacillus cereus (129), B. subtilis (64, 108), E. coli (60, 85), L. lactis (213), L. monocytogenes (14), V. vulnificus (132), Salmonella* Enteritidis *(82),* and *Salmonella* Typhimurium *(32).* Depending on the bacterial species and the extent of the low-temperature stress, up to 50 different CSPs may be expressed *(16).* It is hypothesized that CSPs play a critical role in various cellular and physiological functions, including DNA recombination, transcription, translation, messenger RNA and protein folding, sugar uptake, chemotaxis, and general metabolic efficiency *(63, 86, 212).* Maintenance of membrane fluidity at low temperatures is necessary for cold adaptation and involves at least one constitutively expressed enzyme that is active only at low temperatures *(2).*

Acid tolerance response. Exposure to low pH often induces an acid shock response in bacteria. *Salmonella* Typhimurium can withstand potentially lethal acid shock conditions (pH < 4.0) if previously adapted to milder conditions *(17, 50).* This acid adaptation, stemming from a gradual decrease in pH to nonlethal levels (pH > 4.0), induces the acid tolerance response *(2, 12, 104).* Acid shock responses have also been reported in *L. monocytogenes, E. coli,* and *Shigella flexneri (2),* and can be induced by organic acids commonly used in the food industry *(146).* The more effective acid resistance systems for *E. coli* and *S. flexneri* allow survival at pH 2.0 in the presence of glutamate, while the acid tolerance system for *Salmonella* Typhimurium is functional at pH 3.0 *(2, 11, 52).* During acid adaptation and shock, *Salmonella* Typhimurium induces the expression of at least 60 acid shock proteins in the exponential phase and 48 acid shock proteins in the stationary phase, with five interlapping proteins as determined by proteomic analysis *(11).* These proteins, which are under the control of multiple, overlapping regulatory systems, protect the cell against acid and perhaps other environmental stresses.

Oxidative response. The oxidative response typically involves proteins that prevent (e.g., catalase and superoxide dismutase) and repair (e.g., exonucleases and glycosylases) oxidative damage *(49).* Starvation proteins also have been produced independent of the nutrient for which a cell is starved. Some of these proteins are unique to starvation, while others are common to other stresses *(69, 82, 128, 207).* Starvation and stationary-phase proteins are likely responsible for maintaining long-term survival and enhancing general cellular resistance by stabilizing ribosomes against degradation *(198),* influencing morphological transformations into spherical conformations *(59),* and improving metabolic potential by utilizing alternative growth substrates *(125).*

STRESS-INDUCED CROSS-PROTECTION

Definition of stress-induced cross-protection. Sykes *(195)* attributed bacterial survival in adverse environments to either (i) sublethal treatments that were insufficient to cause death or (ii) an innate means of protection from destructive conditions or treatments. It was inferred from the latter hypothesis that bacterial cells could adapt or acquire resistance to different conditions by modifying metabolic activities, adjusting nutrient utilization, or by using enzymes that were previously present in a recessive role. Although the exact mode of action is not fully understood, a role in stress protection has been proposed for stress proteins, especially HSPs and starvation proteins *(2, 68, 69, 148).* The long-term survival of *E. coli* is dependent on the synthesis of starvation proteins *(125).* Givskov et al. *(59)* concluded that protein synthesis induced by starvation was necessary for *Pseudomonas putida* to develop a general stress-resistant state. Jenkins et al. *(82)* correlated the in-

TABLE 5. *Treatments known to enhance thermotolerance of microorganisms*

Microorganism	Stress treatment	Heat challenge	Reference
Escherichia coli O157:H7	Starvation in distilled water for 24 h at 37°C	56°C for up to 90 min	*174*
	Starvation in 0.85% NaCl (pH 6.6) for 48 h at 37°C	56°C for 50 min	*102*
	Acid adaptation (pH 4.8–4.9) in TSB for 18 h at 37°C	56°C for 50 min	*102*
Nonpathogenic *E. coli*	Starvation in 0.85% NaCl (pH 6.6) for 48 h at 37°C	56°C for 50 min	*102*
	Acid adaptation (pH 4.8–4.9) in TSB for 18 h at 37°C	56°C for 50 min	*102*
	Glucose starvation in M9 minimal media for 4 h at 29°C	57°C	*82*
Salmonella Typhimurium	Starvation in a minimal medium with 0.02% glucose for 10 h at 37°C	52°C	*198*
	Acid adaptation (pH 5.8) in medium E at 37°C	50°C for up to 60 min	*104*
Listeria monocytogenes	Adaptation in tryptose phosphate broth (pH 12.0) at 37°C for 45 min	56°C or 59°C	*196*
	Exposure to 4–8% (vol/vol) ethanol at 35°C for 1 h	56°C	*109*
	Starvation in 0.1 M phosphate buffer (pH 7.0) at 30°C for up to 163 h	56°C	*109*
	Exposure to 500 ppm of H_2O_2 at 35°C for 1 h	56°C	*109*
	Acid adaptation in TSB-YE (pH 4.5) at 35°C for 1 h	56°C	*109*
	Acid shock (pH 4.0) in TSB-YE at room temp for 1 h	58°C	*48*
Pseudomonas putida	Starvation at 30°C by exhaustion of carbon, nitrogen, phosphate or sulfate	47°C	*59*

duction of starvation proteins with protection of *E. coli* against heat and H_2O_2.

Stresses inducing cross-protection. Thermotolerance can be induced by stresses other than heat. Table 5 summarizes the treatments known to induce heat tolerance in various microorganisms. Many sublethal treatments can afford cross-protection against stresses other than heat. Jenkins et al. *(82)* reported that starvation or adaptive treatments with heat, H_2O_2, or ethanol protects *E. coli* against further oxidative (H_2O_2) challenges. According to Zook et al. *(224),* treatment with a commercial 0.1% peroxyacetic acid sanitizer also increased the oxidative tolerance of *E. coli* O157:H7. Starvation of *P. putida* resulted in enhanced resistance to ethanol, heat shock, and osmolarity *(59),* and heat stress has been reported to enhance the survival of *Pseudomonas aeruginosa* to the antibiotic biapenem *(24).* Bang and Drake *(13)* observed that starvation increased freeze–thaw resistance of *V. vulnificus,* while Leenanon and Drake *(102)* observed an increase in freeze-thaw resistance of *E. coli* O157:H7 after both starvation and acid adaptation. According to Bollman et al. *(21),* cold shocking *E. coli* O157:H7 in milk, whole egg, or sausage, but not beef or pork, enhanced survival during frozen storage. Adaptation of *Salmonella* Typhimurium to pH 5.8 reportedly increases tolerance to osmotic (salt) stress, crystal violet, and the lactoperoxidase system *(104).* Lou and Yousef *(110)* found that adaptation of *L. monocytogenes* to 5% (vol/vol) ethanol significantly increased resistance to lethal doses of acid, ethanol, and NaCl. Similarly, adaptation to acid (pH 4.5 to 5.0), ethanol, 500 ppm of H_2O_2, 7% (wt/vol) NaCl, and heat (1 h at 45°C) increased resistance to H_2O_2. Exposure of *L. monocytogenes* to low pH (4.5) has also been shown to provide cross-protection against high hydrostatic pressure and freezing *(209).*

The response to a given stress differs among bacterial species. While Lou and Yousef *(110)* showed that both acid and ethanol adaptation increased the resistance of *L. monocytogenes* to lethal doses of acid, adaptation of *Salmonella* Typhimurium to stresses other than acid did not protect against lethal acid treatment *(101).* Mazzotta *(130)* determined that while acid adaptation at pH 5.0 for 18 to 24 h increased heat resistance at 56, 58, and 60°C for *L. monocytogenes* and *E. coli* O157:H7 as well as two *Salmonella* composites (Gaminara, Rubislaw, and Hartford, and *Salmonella* Typhimurium and Enteritidis) in apple, orange, and white grape juices adjusted to pH 3.9, the increase in heat resistance was greater for *L. monocytogenes* and *E. coli* O157:H7 than it was for *Salmonella.* Furthermore, it has been suggested that gram-negative bacteria are in general more sensitive to cold shock, chilling, and freezing but are more resistant to weak acid preservatives than are gram-positive bacteria *(16).*

The effect of adaptive pretreatments can also vary between different bacterial species and strains. *Campylobacter fetus* has been shown to be more pressure sensitive than *C. jejuni, Campylobacter coli,* and *Campylobacter lari (122).* Cheng et al. *(31)* found that acid adaptation at pH 5.0 for 4 h increased the thermal tolerance of two of three strains of *E. coli* O157:H7 at 52°C. Similarly, starvation was shown to increase the heat resistance of only two of three *E. coli* O157:H7 strains *(174).* In addition, Sharma et al. *(180)* observed that two different acid-adapted serotypes of *Salmonella* responded differently to heating in cantaloupe juice. While acid-adapted cells of *Salmonella* Saphra (97A3312) exhibited a significantly greater $D_{57°C}$-value than did unadapted cells, acid-adapted *Salmonella* Poona (01A3907) did not show an increased resistance over its unadapted counterpart. These observed differences for species and strains may be due to inherent degrees of heat or other stress resistance.

The magnitude and nature of a protective stress response also varies with the treatment. Völker et al. *(207)* found that exposing *B. subtilis* to low salt concentrations was less effective than was exposure to heat shock at inducing thermotolerance. Jenkins et al. *(82)* reported that the oxidative and thermal resistance of nutrient-starved *E. coli* cells was greater than it was for heat-adapted cells, while resistance to H_2O_2 and heat increased proportionally with the length of starvation. Lou and Yousef *(109)* also reported that the level of thermotolerance in *L. monocytogenes* increased proportionately to the severity of each inactivation treatment, including duration of starvation, reduced pH levels, and concentration of ethanol or H_2O_2. Acid shock–induced thermotolerance is also dependent on the type of acid to which a bacterium is exposed. Farber and Pagotto *(48)* observed that, while hydrochloric acid was effective at inducing thermotolerance in *L. monocytogenes,* acetic and lactic acids were not. While cells of *L. monocytogenes* incubated at pH 12.0 for 45 min were significantly more heat resistant at 56°C than were those incubated at pH 7.3, cells incubated at pH 9.0, 10.0, or 11.0 did not exhibit this enhanced thermotolerance, although the strength of the agent used to adjust the pH had an effect on thermotolerance *(196).*

BACTERIAL STRESS AND VIRULENCE

The presence of injured microorganisms in food poses significant public health concerns. Injured cells may initially go undetected during routine quality control checks and at critical control points during manufacturing. However, subsequent cellular repair in the food may allow for growth and the ensuing results, including spoilage and the production of toxins and other virulence factors *(83).* As an example, three virulence factors of *E. coli* O157:H7, verotoxins 1 and 2, and the attaching and effacing gene, *eae,* were retained after starvation and heat stress *(174).* According to Singh and McFeters *(181),* virulence of *Yersinia enterocolitica* in orally inoculated mice also was unaffected by chlorine stress. A bacterium's pathogenicity or virulence may be considered the end result of its ability to repair injury *(41).* Mekalanos *(134)* defines *virulence determinants* as those factors contributing to infection and disease, but not to general "housekeeping" functions. A clear line of distinction is not always seen between the two, but virulence genes, to some extent, are part of an adaptive response to stresses encountered in a host *(78).* Many of the stresses that are intrinsically part of a host's defense system are similar to those encountered in the natural environment (Fig. 1). Pathogenic microorganisms may see exposure to stress in both natural environments and food processing facilities as a signal for the expression of virulence factors *(110).* A strain of *Salmonella* Enteritidis possessing enhanced acid and heat tolerance was shown to be more virulent for mice and more invasive for chickens than was a nonresistant reference strain *(71).*

Expression of many virulence factors depends on environmental cues *(96, 134).* Several environmental conditions have been identified that induce expression of Spv (*Salmonella* plasmid virulence) proteins, including glucose

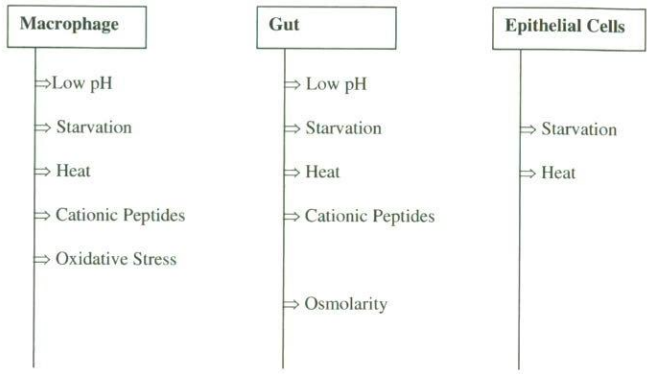

FIGURE 1. *Environmental stresses imposed on bacterial pathogens by host defense mechanisms (53).*

starvation, low pH, elevated temperature, and iron limitation *(35, 53, 203).* The *spv* genes in several serovars of *Salmonella* (e.g., Typhimurium, Dublin, and Enteritidis) are thought to facilitate rapid multiplication in host cells, systemic spread, and infection of extraintestinal tissues *(35).* An invasion gene in *Salmonella* Typhimurium, *invA,* is reportedly induced by high osmolarity *(8, 134)* and expression of listeriolysin, a major virulence factor in *L. monocytogenes,* by heat shock, oxidative stress, and transition to the stationary phase *(37, 134, 147, 186, 187).* Production of thermostable direct hemolysin, a major virulence factor of *V. parahaemolyticus,* is enhanced by heat shock at 42°C *(211).*

Temperature-regulated virulence factors have been identified in enteroinvasive *E. coli (19), S. flexneri (44, 128, 134), L. monocytogenes (54, 103, 128), Y. enterocolitica (182),* and heat shock has been linked to virulence in *L. monocytogenes (8, 134), Salmonella* Typhimurium *(8, 53),* and *Shigella* spp. *(194).* As pathogens traverse from the natural environment, through contaminated food, water, or insect vectors into mammalian hosts, a sudden increase in body temperature triggers strong heat shock–like responses that intensify when host defense mechanisms (including fever) are encountered *(105).*

The intracellular pathogen *Y. enterocolitica* undergoes a global stress response to the hostile environment encountered in host macrophages, including the induction of heat shock proteins *(216). Salmonella* Typhimurium also expresses several stress proteins in the host macrophage, including HSPs *(3, 25, 53, 176).* To a certain extent, HSP synthesis appears to protect pathogens from host defense mechanisms, and HSPs themselves may be considered ubiquitous evasion factors of pathogens *(91).* Heat shock proteins are often dominant antigens of the host immune response, perhaps because of their wide distribution and homology among different species *(25, 105, 225).* Host cells can also synthesize HSPs, and an extended assault on the immune system with HSP antigens that are similar in the host and infecting pathogens may promote autoimmune disease *(91, 225).*

Acid tolerance is thought to enhance virulence in one or both of the following ways: (i) resistance to strong acid conditions facilitates survival in the stomach, thereby decreasing the requisite infective dose *(219, 220, 221),* and

(ii) resistance to moderately acidic conditions improves pathogen survival in acidic foods dependent on low pH for microbial inactivation *(102).* Acid tolerance of *E. coli* O157:H7 likely contributes to its low infective dose. Acid-sensitive strains of *Salmonella* Typhimurium exhibit reduced virulence *(50),* whereas acid-tolerant mutants of *L. monocytogenes* exhibit increased virulence in the mouse model *(146).* Disruption of the RpoS system in *Salmonella,* which is involved in acid and general stress tolerance, may offer insight into the relationship between stress and virulence. *rpoS* null mutants are attenuated for mice after both oral and intraperitoneal infection *(176).* For many pathogens, acid tolerance seems to enhance survival in the host macrophage *(8, 54).*

Due to limited oxygen availability in the small intestine, anaerobic stress is hypothesized to enhance virulence of pathogens invading the gastrointestinal tract *(89).* Anaerobiosis can reportedly increase the invasiveness of *Salmonella* Typhimurium as confirmed by the restricted invasion of aerobically grown cells *(53, 134).* Exposure of *Salmonella* Typhi to anaerobic conditions also enhances virulence *(89).* Osmotic stress may prepare microorganisms for survival within a host, as the expression of some virulence factors may be enhanced in the osmolarity range of host tissues. Osmoregulation of virulence has been noted for several microorganisms, including *Salmonella* Typhimurium, *S. flexneri,* and *Vibrio cholerae (134, 183).*

The preceding examples indicate that alterations in cellular physiology, including stress protein synthesis in response to environmental stresses, may strongly impact virulence. An extension of this is the purported role of alternative sigma factors (e.g., σ^B) in the regulation of virulence factors *(92, 93).* A bacterium's ability to successfully handle environmental stress partially defines its virulence, since the response to such stress often includes the expression and control of various virulence factors *(8).* These consequences led Archer *(8)* to question whether a "reduction in preservation might not in fact lead to a reduction in the immediate virulence of certain pathogens, and, additionally, to a lowering of the rate of emergence of new or better host-adapted pathogens."

THE ROLE OF REACTIVE OXYGEN INTERMEDIATES IN BACTERIAL INJURY AND STRESS RESPONSE

The significance of reactive oxygen intermediates in bacterial injury. The role of reactive oxygen intermediates in bacterial injury and stress response is becoming more apparent. Radical chemical species may injure cells by oxidation of membranes, periplasmic targets, enzymes, proteins, DNA, and iron-sulfur clusters. Other non–radical reactive oxygen species (ROS), including singlet oxygen, ozone, lipid peroxides, and hypochlorous acid, may also injure cells *(202).* The efficacy of antimicrobial processing interventions such as UV light, gamma radiation, nonthermal plasma treatment, and thermal processing, along with desiccation, freezing, and the application of hypochlorous acid and other antimicrobials is intertwined with the generation of reactive oxygen and reactive chemical species. Effective recovery and detection of injured bacteria in food matrices has much to do with overcoming the effects of ROS that are lethal to cellular components and metabolic processes. In fact, compounds are frequently added to bacterial recovery media to overcome the toxic effects of ROS (e.g., sodium pyruvate, 3′3′-thiodiopropionic acid, catalase, superoxide dismutase, and Oxyrase) *(7, 9, 29, 34, 62, 119, 120, 121, 123, 190).* Further, in order for a foodborne pathogen to present illness in a host, the organism must be able to withstand the cascade of ROS that it will encounter not only in the food matrix, but also within the host's gastrointestinal tract and eukaryotic cells.

Nitric oxide and Fenton reaction–mediated hydroxyl radicals. Nitric oxide is one example of a ROS that bacteria frequently encounter. Nitric oxide can be converted to lethal peroxynitrite on reaction with the superoxide anion as by-products of respiration or during the invasion of eukaryotic host cells. Superoxide is not known to oxidize DNA and therefore has limited bactericidal activity; however, it is implicated in the generation of a more lethal ROS via Fenton reaction–mediated iron-superoxide toxicity *(199)* illustrated in the reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + HO^{-}$. When bacteria enter a host macrophage, a respiratory burst of superoxide occurs, generating hydrogen peroxide. In the presence of free iron via the Fenton reaction, H_2O_2 is catalyzed into the short-lived hydroxyl radical, the most reactive of the oxygen species, which can oxidize any cellular macromolecule to which it attaches *(199).*

Protections against ROS damage. *E. coli* is known to protect against ROS damage by the expression of at least 112 genes, on exposure to paraquat-generated superoxide *(155),* and by the expression of at least 140 genes in the presence of hydrogen peroxide *(223).* The *fur* regulatory protein in *E. coli* controls numerous genetic responses, including regulation of cytoplasmic iron, while the RecA protein facilitates maintenance and repair of damaged DNA. Although iron is indispensable to cellular metabolism, excesses of free iron within the bacterial cell lead to increases in ROS and bacterial injury. The deleterious effects of free iron have been demonstrated in studies using Δ*fur* mutants of *E. coli* that are unable to downregulate intracellular levels of iron, resulting in oxidative DNA damage *(200).* When actively growing cultures of *E. coli* were transferred from anaerobic to aerobic incubation, Δ*fur* mutants as well as Δ*recA* mutants continued multiplying, while bacteria lacking both the Δ*fur* and Δ*recA* genes experienced a >2.5-log reduction in population within 3 h, punctuating the importance of both genes in resuscitation from oxidative damage. Research has shown that chelation of excess iron by compounds such as ferrozine can prevent cellular injury that would normally be sustained by exposure of up to 2.5 mM H_2O_2 for 3 min *(38, 78, 79, 152).* Furthermore, bacteria can allay oxidative injury by overproduction of a ferritin-like iron scavenger by Dps protein induction when exposed to H_2O_2 *(77).* Bacterial survival genes must therefore maintain iron homeostasis to promote metabolic activities,

yet simultaneously prevent an overabundance of free iron that will lead to oxidative damage.

Intervention strategies utilizing ROS. Many microbial reduction strategies used in the food industry may be bacteriostatic or bactericidal to foodborne pathogens, due to the promotion of intra- or extracellular ROS. Induction of RpoS (alternate sigma factor) in *Salmonella* typically coincides with entrance into the stationary phase and a concomitant drop in oxidation-reduction potential. Komitopoulou et al. *(98),* however, reported an irreversible downregulation of RpoS expression in *Salmonella* Typhimurium that was grown under high oxygen tension, resulting in significantly more heat-sensitive cells than those incubated under ambient atmospheric conditions. Conversely, artificially lowering the oxidation-reduction potential of the *Salmonella* growth medium resulted in premature entry into stationary phase, induction of RpoS, and bacterial resistance. Designing food processes that maintain elevated oxygen tension might inhibit RpoS induction, thereby sensitizing contaminating *Enterobacteriaceae* to thermal, chemical, electrical, physical, desiccation, gastrointestinal, or other stresses to which bacteria would otherwise be more resistant. While more effective microbial reduction strategies may be possible if microbial genetic responses are considered, this approach is complicated by variations within bacterial genera. As an example of variation within the family *Enterobacteriaceae,* more than 2,500 serovars have been identified within the genus *Salmonella,* including 50 phage types of *S. enterica* serovar Enteritidis alone *(72, 156, 157).*

The bactericidal activity of sodium hypochlorite or NaOCl (e.g., household bleach—one of the most widely used disinfectants in food processing) is attributed to the release of hypochlorous acid (HOCl) in the following reaction: $NaOCl + H_2O \leftrightarrow NaOH + HOCl$. The antibacterial activity of NaOCl is closely related to the action of reactive oxygen intermediates, possibly due to HOCl inactivation of antioxidants such as superoxide dismutase, catalase, and peroxidase in vivo and in vitro *(9, 29, 123).* Other indications that ROS plays a role in the antibacterial activity of HOCl include increased recovery of HOCl-injured cells under anaerobiosis versus an increase in HOCl toxicity on exposure to oxygen *(46),* expression of the ROS-defense regulon *soxRS* after HOCl injury in *E. coli (46),* and the increased production of Fenton reaction–mediated hydroxyl radicals by iron on exposure to HOCl *(172).* Maalej et al. *(112)* demonstrated that prior exposure to HOCl resulted in upregulation of the *perR* regulon and subsequent resistance to superoxide in *S. aureus.*

Oxygen tension and reactive oxygen intermediates have also been implicated in injury or death when bacterial cells are exposed to desiccation, freezing, and thermal stress, whereas antioxidants will promote resuscitation of cells injured under these conditions *(18, 62, 112, 119, 120, 131).* Dodd et al. *(43)* reviewed the literature pertaining to the suicide response theory and its relationship to oxygen tension and ROS. This theory maintains that when pathogens experience stress, bacterial multiplication ceases; however, aerobic cellular metabolism and the uncoupling of energy persist, releasing a lethal burst of free radicals within the cytoplasm. This helps explain why metabolically active *Salmonella* and *E. coli* in the logarithmic phase of growth are more susceptible to stress than are cells in the stationary phase, which exhibit diminished metabolic activity and an expressed *rpoS.* These claims are bolstered by a study employing electron spin resonance spectroscopy, which demonstrated that after ethanol injury, respiratory bacteria, unlike strictly anaerobic microorganisms, produced a cellular burst of ROS *(4).* While induction of RpoS partially explains the resistance of stationary phase cells to inimical processes, Dodd et al. *(43)* suggest that recalcitrance to stress under some conditions is a physiological rather than an RpoS-regulated genetic response. This was demonstrated in studies where *Enterobacteriaceae,* which were exposed to decreased levels of dissolved oxygen, were summarily resistant to injurious conditions, whereas RpoS induction did not occur for at least 2 h more *(5, 6, 45).* These findings support the suggestion that higher oxygen tension foods can potentially sensitize bacteria to hurdle technology in the form of lethal processing or environmental conditions. Additionally, this understanding of the genetic basis for bactericidal or bacteriostatic effects of a controlled-oxygen environment may have important implications when designing active and modified atmosphere packaging systems.

Resuscitation of ROS-injured bacteria. Selection of appropriate microbiological enrichment and resuscitation media is critical to the laboratory detection and recovery of injured cells. The suitability of media for recovery of injured cells is also related to the presence of reactive oxygen intermediates in recovery agars or broths, which are known to contain up to 10 μM of H_2O_2 generated by the oxidation of sugars during high-temperature sterilization *(80).* Stephens et al. *(190)* reported that when 12 different peptones were compared for cellular resuscitation, up to 3.5 log CFU/ml more heat-injured salmonellae were recovered by a most-probable-number method for the best versus the worst formulations. In fact, whereas one peptone resulted in ca. 4-log CFU/ml reduction in *Salmonella* after thermal stress, almost no reduction was detected when the best performing peptone was supplemented with 2% Oxyrase, a commercially available oxygen tension reducer. The study also found that ROS and reduced bacterial resuscitation occurred commensurate with increasing concentrations of reducing sugars and riboflavin, due to auto-oxidation of sugars during thermal sterilization and photosensitization of riboflavin. The authors recommended that in order to maximize the benefits of protective reducing compounds in recovery media, these recovery supplements in all likelihood should be added during media preparation and early storage, when most toxic components are generated.

CONCLUDING REMARKS

The microbial response to stress is not "all or none" with exposure to adverse environmental conditions (e.g., heat, cold, acid, alkali, starvation, and/or osmotic stress) inducing various degrees of injury depending on the physiological state of individual cells within the population. The

percentage of "healthy" and "injured" cells in such a population has been historically based on the organisms' ability to form visible colonies on selective versus nonselective media, with the outcome largely dictated by media composition and incubation temperature. Bacteria typically respond to stress by altering their cellular morphology, membrane composition, cellular metabolism, and degree of virulence. Such "stress-hardened" organisms produce a range of stress proteins that frequently afford cross-protection against other stresses. The presence of injured cells in a food can pose major public health concerns, since many bacterial pathogens can become more resistant to cooking and other commonly used microbial reduction strategies as a result of sublethal injury. Subsequent repair of injured cells in a food and restoration of virulence if previously lost requires a complex series of biochemical events that will differ based on the food product as well as the type and degree of injury. However, many injured pathogens either retain or exhibit enhanced virulence in foods, thus making their detection crucial to safeguard the food supply.

REFERENCES

1. Abbis, J. S. 1983. Injury and resuscitation of microbes with reference to food microbiology. *Irish J. Food Sci. Technol.* 7:69–81.
2. Abee, T., and J. A. Wouters. 1999. Microbial stress response in minimal processing. *Int. J. Food Microbiol.* 50:65–91.
3. Abshire, K. Z., and F. C. Neidhardt. 1993. Analysis of proteins synthesized by *Salmonella typhimurium* during growth within a host macrophage. *J. Bacteriol.* 175:3734–3743.
4. Aldsworth, T. G., C. E. R. Dodd, and G. S. A. B. Stewart. 1998. Bacterial self destruction—the "suicide response" explored, p. 263–266. *In* A. Roda, M. Pazzagli, L. J. Kricka, and P. E. Stanley (ed.), Bioluminescence and chemiluminescence: perspectives for the 21st century. John Wiley and Sons, Chichester, UK.
5. Aldsworth, T. G., R. L. Sharman, and C. E. R. Dodd. 1999. Bacterial suicide through stress. *Cell. Mol. Life Sci.* 55:378–383.
6. Aldsworth, T. G., R. L. Sharman, C. E. R. Dodd, and G. S. A. B. Stewart. 1998. A competitive microflora increases the resistance of *Salmonella typhimurium* to inimical processes: evidence for a suicide response. *Appl. Environ. Microbiol.* 64:1323–1327.
7. Andrews, G. P., and S. E. Martin. 1979. Catalase activity during recovery of heat-stressed *Staphylococcus aureus* MF-31. *Appl. Environ. Microbiol.* 38:390–394.
8. Archer, D. L. 1996. Preservation microbiology and safety: evidence that stress enhances virulence and triggers adaptive mutations. *Trends Food Sci. Technol.* 7:91–95.
9. Aruoma, O. I., and B. Halliwell. 1987. Action of hypochlorous acid on the antioxidant protective enzymes superoxide dismutase, catalase and glutathione peroxidase. *Biochem. J.* 248:973–976.
10. Asakura, H., S.-I. Makino, T. Takagi, A. Kuri, T. Kurazano, M. Watarai, and T. Shirahata. 2002. Passage in mice causes a change in the ability of *Salmonella enterica* serovar Oranienburg to survive NaCl osmotic stress: resuscitation from the viable but non-culturable state. *FEMS Microbiol. Lett.* 212:87–93.
11. Audia, J. P., C. C. Webb, and J. W. Foster. 2001. Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. *Int. J. Med. Microbiol.* 291:97–106.
12. Bang, I. S., J. P. Audia, Y. K. Park, and J. W. Foster. 2002. Autoinduction of the *ompR* response regulator by acid shock and control of the *Salmonella enterica* acid tolerance response. *Mol. Microbiol.* 44:1235–1250.
13. Bang, W., and M. A. Drake. 2002. Resistance of cold- and starvation-stressed *Vibrio vulnificus* to heat and freeze–thaw exposure. *J. Food Prot.* 65:975–980.
14. Bayles, D. O., B. A. Annous, and B. J. Wilkinson. 1996. Cold stress proteins induced in *Listeria monocytogenes* in response to temperature downshock and growth at low temperatures. *Appl. Environ. Microbiol.* 62:1116–1119.
15. Baylis, C. L., S. MacPhee, and R. P. Betts. 2000. Comparison of two commercial preparations of buffered peptone water for the recovery and growth of *Salmonella* bacteria from foods. *J. Appl. Microbiol.* 89:501–510.
16. Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comp. Rev. Food Sci.* 3:1–20.
17. Bearson, S. M., W. H. Benjamin, Jr., W. E. Swords, and J. W. Foster. 1996. Acid shock induction of RpoS is mediated by the mouse virulence gene *mviA* of *Salmonella typhimurium*. *J. Bacteriol.* 178: 2572–2579.
18. Benov, L., and I. Fridovich. 1995. Superoxide dismutase protects against aerobic heat shock in *Escherichia coli*. *J. Bacteriol.* 177: 3344–3346.
19. Berlutti, F., M. Casalino, C. Zagaglia, P. A. Fradiani, P. Visca, and M. Nicoletti. 1998. Expression of the virulence plasmid-carried apyrase gene (*apy*) of enteroinvasive *Escherichia coli* and *Shigella flexneri* is under the control of H-NS and the VirF and VirB regulatory cascade. *Infect. Immun.* 66:4957–4964.
20. Beuchat, L. R. 1978. Injury and repair of gram-negative bacteria, with special consideration of the involvement of the cytoplasmic membrane. *Adv. Appl. Microbiol.* 23:219–243.
21. Bollman, J., A. Ismond, and G. Blank. 2001. Survival of *Escherichia coli* O157:H7 in frozen foods: impact of the cold shock response. *Int. J. Food Microbiol.* 64:127–138.
22. Boziaris, I. S., and M. R. Adams. 2001. Temperature shock, injury and transient sensitivity to nisin in gram negatives. *J. Appl. Microbiol.* 91:715–724.
23. Brashears, M. M., A. Amezquita, and J. Stratton. 2001. Validation of methods used to recover *Escherichia coli* O157:H7 and *Salmonella* spp. subjected to stress conditions. *J. Food Prot.* 64:1466–1471.
24. Bremer, E., and R. Krämer. 2000. Coping with osmotic challenges: osmoregulation through accumulation and release of compatible solutes, p. 79–97. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, DC.
25. Buchmeier, N. A., and F. Heffron. 1990. Induction of *Salmonella* stress proteins upon infection of macrophages. *Science* 248:730–732.
26. Bunning, V. K., R. G. Crawford, J. T. Tierney, and J. T. Peeler. 1990. Thermotolerance of *Listeria monocytogenes* and *Salmonella typhimurium* after sublethal heat shock. *Appl. Environ. Microbiol.* 56:3216–3219.
27. Busta, F. F. 1976. Practical implications of injured microorganisms in food. *J. Milk Food Technol.* 39:138–145.
28. Busta, F. F. 1994. Importance of injured food-borne microorganisms in minimal processing, p. 227–231. *In* R. P. Singh and F. A. R. Oliveira (ed.), Minimal processing of foods and process optimization: an interface. CRC Press, Boca Raton, FL.
29. Calabrese, J. P., and G. K. Bissonnette. 1990. Improved membrane filtration method incorporating catalase and sodium pyruvate for detection of chlorine-stressed coliform bacteria. *Appl. Environ. Microbiol.* 56:3558–3564.
30. Castillo, A., L. M. Lucia, I. Mercado, and G. R. Acuff. 2001. In-plant evaluation of a lactic acid treatment for reduction of bacteria on chilled beef carcasses. *J. Food Prot.* 64:738–740.
31. Cheng, H.-Y., H.-Y. Yang, and C.-C. Chou. 2002. Influence of acid adaptation on the tolerance of *Escherichia coli* O157:H7 to some subsequent stresses. *J. Food Prot.* 65:260–265.
32. Craig, J. E., D. Boyle, K. P. Francis, and M. P. Gallagher. 1998. Expression of the cold-shock gene *cspB* in *Salmonella typhimurium* occurs below a threshold temperature. *Microbiology* 144:697–704.
33. Czechowicz, S. M., O. Santos, and E. A. Zottola. 1996. Recovery of thermally stressed *Escherichia coli* O157:H7 by media supplemented with pyruvate. *Int. J. Food Microbiol.* 33:275–284.
34. Dallmier, A. W., and S. E. Martin. 1988. Catalase and superoxide dismutase activities after heat injury of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 54:581–582.

35. D'Aoust, J.-Y. 1997. *Salmonella* species, p. 129–158. *In* M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
36. D'Aoust, J.-Y., D. W. Warburton, and A. M. Sewell. 1985. *Salmonella* and food safety. *J. Food Prot.* 48:1062–1066.
37. Datta, A. R., and M. H. Kothary. 1993. Effects of glucose, growth temperature, and pH on listeriolysin O production in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 59:3495–3497.
38. de Mello Filho, A. C., and R. Meneghini. 1985. Protection of mammalian cells by *o*-phenanthroline from lethal and DNA-damaging effects produced by active oxygen species. *Biochim. Biophys. Acta* 847:82–89.
39. Dickson, J. S., and J. F. Frank. 1993. Bacterial starvation stress and contamination of beef. *Food Microbiol.* 10:215–222.
40. Dickson, J. S., and G. R. Siragusa. 1994. Survival of *Salmonella typhimurium, Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A during storage on beef sanitized with organic acids. *J. Food Saf.* 14:313–327.
41. Dillon, J. R., and G. S. Bezanson. 1984. Microbial response to injuries from host defense mechanisms and antibiotics, p. 187–215. *In* A. Hurst and A. Nasim (ed.), Repairable lesions in microorganisms. Academic Press, London.
42. Dodd, C. E., and T. G. Aldsworth. 2002. The importance of RpoS in the survival of bacteria through food processing. *Int. J. Food Microbiol.* 74:189–194.
43. Dodd, C. E. R., P. J. Richards, and T. G Aldsworth. 2007. Suicide through stress: a bacterial response to sub-lethal injury in the food environment. *Int. J. Food Microbiol.* 120:46–50.
44. Dorman, C. J., N. N. Bhriain, and C. F. Higgins. 1990. DNA supercoiling and environmental regulation of virulence gene expression in *Shigella flexneri. Nature* 344:789–792.
45. Duffy, G., A. Ellison, W. Anderson, M. B. Cole, and G. S. A. B. Steward. 1995. The use of bioluminescence to model the thermal inactivation of *Salmonella typhimurium* in the presence of a competitive microflora. *Appl. Environ. Microbiol.* 61:3463–3465.
46. Dukan, S., S. Dadon, D. R. Smulski, and S. Belkin. 1996. Hypochlorous acid activates the heat shock and soxRS systems of *Escherichia coli. Appl. Environ. Microbiol.* 62:4003–4008.
47. Farber, J. M., and B. E. Brown. 1990. Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. *Appl. Environ. Microbiol.* 56:1584–1587.
48. Farber, J. M., and F. Pagotto. 1992. The effect of acid shock on the heat resistance of *Listeria monocytogenes. Lett. Appl. Microbiol.* 15:197–201.
49. Farr, S. B., and T. Kogoma. 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium. Microbiol. Rev.* 55: 561–585.
50. Foster, J. W. 1995. Low pH adaptation and the acid tolerance response of *Salmonella typhimurium. Crit. Rev. Microbiol.* 21:215–237.
51. Foster, J. W. 2000. Microbial responses to acid stress, p. 99–115. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, DC.
52. Foster, J. W., and M. Moreno. 1999. Inducible acid tolerance mechanisms in enteric bacteria. *Novartis Found. Symp.* 221:55–69.
53. Foster, J. W., and M. P. Spector. 1995. How *Salmonella* survive against the odds. *Annu. Rev. Microbiol.* 49:145–174.
54. Gahan, C. G. M., and C. Hill. 1999. The relationship between acid stress responses and virulence in *Salmonella typhimurium* and *Listeria monocytogenes. Int. J. Food Microbiol.* 50:93–100.
55. Genthner, F. J., and S. E. Martin. 1990. Ribosome assembly in *Salmonella typhimurium* 7136 during recovery from heat shock. *Food Microbiol.* 7:53–67.
56. Gerris, C. V. 1 April 2000. Use of lactic acid for carcass decontamination. *Meat Poult.*
57. Gilbert, P. 1984. The revival of microorganisms sublethally injured by chemical inhibitors, p. 175–197. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
58. Girgis, H. S., J. Smith, J. B. Luchansky, and T. R. Klaenhammer. 2003. Stress adaptations of lactic acid bacteria, p. 159–211. *In* A. E. Yousef and V. J. Juneja (ed.), Microbial stress adaptation and food safety. CRC Press, Boca Raton, FL.
59. Givskov, M., L. Eberl, S. Møller, L. K. Poulsen, and S. Molin. 1994. Responses to nutrient starvation in *Pseudomonas putida* KT2442: analysis of general cross-protection, cell shape, and macromolecular content. *J. Bacteriol.* 176:7–14.
60. Goldstein, J., N. S. Pollitt, and M. Inouye. 1990. Major cold-shock protein of *Escherichia coli. Proc. Natl. Acad. Sci. USA* 87:283–287.
61. Gould, G. W. 1984. Injury and repair mechanisms in bacterial spores, p. 199–220. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
62. Gram, L., P. Pederson, and H. Sogaard. 1984. An evaluation of the effect of catalase and 3′3′-thiodipropionic acid on the recovery of freeze-injured coliform bacteria. *Int. J. Food Microbiol.* 1:155–162.
63. Graumann, P., and M. A. Marahiel. 1996. Some like it cold: response of microorganisms to cold shock. *Arch. Microbiol.* 166:293–300.
64. Graumann, P., K. Schröder, R. Schmid, and M. A. Marahiel. 1996. Cold shock stress-induced proteins in *Bacillus subtilis. J. Bacteriol.* 178:4611–4619.
65. Guillier, L., P. Pardon, and J.-C. Augustin. 2005. Influence of stress on individual lag time distributions of *Listeria monocytogenes. Appl. Environ. Microbiol.* 71:2940–2948.
66. Gurtler, J. G., and L. R. Beuchat. 2005. Performance of media for recovering stressed cells of *Enterobacter sakazakii* as determined using spiral plating and ecometric techniques. *Appl. Environ. Microbiol.* 71:7661–7669.
67. Hartmann, M., A. Barsch, K. Niehaus, A. Puhler, A. Tauch, and J. Kalinowski. 2004. The glycosylated cell surface protein Rpf2, containing a resuscitation-promoting factor motif, is involved in intercellular communication of *Corynebacterium glutamicum. Arch. Microbiol.* 182:299–312.
68. Hecker, M., W. Schumann, and U. Völker. 1996. Heat-shock and general stress response in *Bacillus subtilis. Mol. Microbiol.* 19:417–428.
69. Hengge-Aronis, R. 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli. Cell* 72: 165–168.
70. Hengge-Aronis, R. 2000. The general stress response in *Escherichia coli,* p. 161–178. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, DC.
71. Humphrey, T. 2004. *Salmonella,* stress response and food safety. *Nature Rev.* 2:504–509.
72. Humphrey, T. J., A. Williams, K. McAlpine, M. S. Lever, J. Guard-Petter, and J. M. Cox. 1996. Isolates of *Salmonella enterica* Enteritidis PT4 with enhanced heat and acid tolerance are more virulent in mice and more invasive in chickens. *Epidemiol. Infect.* 177:79–88.
73. Hurst, A. 1977. Bacterial injury: a review. *Can. J. Microbiol.* 23: 935–944.
74. Hurst, A. 1984. Revival of vegetative bacteria after sublethal heating, p. 77–103. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
75. Hurst, A., G. S. Hendry, A. Hughes, and B. Paley. 1976. Enumeration of sublethally heated *Staphylococci* in some dried foods. *Can. J. Microbiol.* 22:677–683.
76. Hurst, A., and A. Hughes. 1981. Repair of salt tolerance and repair of lost D-alanine and magnesium following sublethal heating of *Staphylococcus aureus* are independent events. *Can. J. Microbiol.* 27:627–632.
77. Ilari, A., P. Ceci, D. Ferrari, G. L. Rossi, and E. Chiancone. 2002. Iron incorporation into *Escherichia coli* Dps gives rise to a ferritin-like microcrystalline core. *J. Biol. Chem.* 277:37619–37623
78. Imlay, J. A. 2003. Pathways of oxidative damage. *Ann. Rev. Microbiol.* 57:395–418.
79. Imlay, J. A., and S. Linn. 1988. Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. *Science* 240:640–642.

80. Institute of Food Technologists. 2002. Expert report on emerging microbiological food safety issues. Implications for control in the 21st century. Institute of Food Technologists, International Food Safety and Quality Conference and Exposition, Atlanta, 20 February 2002.
81. Jeffreys, A. G., K. M. Hak, R. J. Steffan, J. W. Foster, and A. K. Bej. 1998. Growth, survival and characterization of *cspA* in *Salmonella enteritidis* following cold shock. *Curr. Microbiol.* 36:29–35.
82. Jenkins, D. E., J. E. Schultz, and A. Matin. 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli. J. Bacteriol.* 170:3910–3914.
83. Johnson, K. M., and F. F. Busta. 1984. Detection and enumeration of injured bacterial spores in processed foods, p. 241–256. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
84. Jones, P. G., M. Mitta, Y. Kim, W. Jiang, and M. Inouyi. 1996. Cold shock induces a major ribosomal-associated protein that unwinds double-stranded RNA in *Escherichia coli. Proc. Natl. Acad. Sci. USA* 93:76–80.
85. Jones, P. G., R. VanBogelen, and F. C. Neidhardt. 1987. Induction of proteins in response to low temperature in *Escherichia coli. J. Bacteriol.* 169:2092–2095.
86. Juneja, V. K., P. G. Klein, and B. S. Marmer. 1998. Heat shock and thermotolerance of *Escherichia coli* O157:H7 in a model beef gravy system and ground beef. *J. Appl. Microbiol.* 84:677–684.
87. Juneja, V. K., and J. S. Novak. 2003. Adaptation of foodborne pathogens to stress from exposure to physical intervention strategies, p. 31–53. *In* A. E. Yousef and V. J. Juneja (ed.), Microbial stress adaptation and food safety. CRC Press, Boca Raton, FL.
88. Kang, D. H., and G. R. Siragusa. 1999. Agar underlay method for recovery of sublethally heat-injured bacteria. *Appl. Environ. Microbiol.* 65:5334–5337.
89. Kapoor, S., R. D. Singh, P. C. Sharma, and M. Khullar. 2002. Anaerobiosis induced virulence of *Salmonella typhi. Indian J. Med. Res.* 115:184–188.
90. Kaprelyants, A. S., G. V. Mukamolova, H. M. Davey, and D. B. Kell. 1996. Quantitative analysis of the physiological heterogeneity within starved cultures of *Micrococcus luteus* by flow cytometry and cell sorting. *Appl. Environ. Microbiol.* 62:1311–1316.
91. Kaufmann, S. H. 1989. Stress proteins: virulence factors of intracellular disease agents? *Immun. Infekt.* 17:124–128.
92. Kazmierczak, M. J., S. C. Mithoe, K. J. Boor, and M. Wiedmann. 2003. *Listeria monocytogenes* sigma B regulates stress response and virulence functions. *J. Bacteriol.* 185:5722–5734.
93. Kazmierczak, M. J., M. Wiedmann, and K. J. Boor. 2005. Alternative sigma factors and their roles in bacterial virulence. *Microbiol. Mol. Biol. Rev.* 69:527–543.
94. Kell, D. B., and M. Young. 2000. Bacterial dormancy and culturability: the role of autocrine growth factors. *Curr. Opin. Microbiol.* 3:238–243.
95. Kennedy, J. E., J. L. Oblinger, and R. L. West. 1980. Fate of *Salmonella infantis, Staphylococcus aureus,* and *Hafnia alvei* in vacuum packaged beef plate pieces during refrigerated storage. *J. Food Sci.* 45:1273–1277, 1300.
96. Knøchel, S., and S. Gould. 1995. Preservation microbiology and safety: quo vadis? *Trends Food Sci. Technol.* 6:127–131.
97. Kobayashi, H., T. Miyamoto, Y. Hashimoto, M. Kiriki, A. Motomatsu, K. I. Honjoy, and M. Ilo. 2005. Identification of factors involved in recovery of heat-injured *Salmonella* Enteritidis. *J. Food Prot.* 68:932–941.
98. Komitopoulou, E., N. J. Bainton, and M. R. Adams. 2004. Oxidation-reduction potential regulates RpoS levels in *Salmonella* Typhimurium. *J. Appl. Microbiol.* 96:271–278.
99. Lazazzera, B. A. 2000. Quorum sensing and starvation: signals for entry into stationary phase. *Curr. Opin. Microbiol.* 3:177–182.
100. LeClerc, J. E., B. Li, W. L. Payne, and T. A. Cebula. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* 274:1208–1211
101. Lee, L. S., J. Lin, H. K. Hall, B. Bearson, and J. W. Foster. 1995. The stationary-phase sigma factor σ^S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium. Mol. Microbiol.* 17:155–167.
102. Leenanon, B., and M. A. Drake. 2001. Acid stress, starvation, and cold stress affect poststress behavior of *Escherichia coli* O157:H7 and nonpathogenic *Escherichia coli. J. Food Prot.* 64:970–974.
103. Leimeister-Wachter, M., E. Domann, and T. Chakraborty. 1992. The expression of virulence genes in *Listeria monocytogenes* is thermoregulated. *J. Bacteriol.* 174:947–952.
104. Leyer, G. J., and E. A. Johnson. 1993. Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium. Appl. Environ. Microbiol.* 59:1842–1847.
105. Lindquist, S. 1992. Heat-shock proteins and stress tolerance in microorganisms. *Curr. Opin. Gen. Develop.* 2:748–755.
106. Linton, R. H., J. B. Webster, M. D. Pierson, J. R. Bishop, and C. R. Hackney. 1992. The effect of sublethal heat shock and growth atmosphere on the heat resistance of *Listeria monocytogenes* ScottA. *J. Food Prot.* 55:84–87.
107. Loewen, P. C., B. Hu, J. Strutinsky, and R. Sparling. 1998. Regulation in the *rpoS* regulon of *Escherichia coli. Can. J. Microbiol.* 44:707–717.
108. Lottering, E. A., and U. N. Streips. 1995. Induction of cold shock proteins in *Bacillus subtilis. Curr. Microbiol.* 30:193–199.
109. Lou, Y., and A. E. Yousef. 1996. Resistance of *Listeria monocytogenes* to heat after adaptation to environmental stresses. *J. Food Prot.* 59:465–471.
110. Lou, Y., and A. E. Yousef. 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Appl. Environ. Microbiol.* 63:1252–1255.
111. Lucht, L., G. Blank, and J. Borsa. 1998. Recovery of foodborne microorganisms from potentially lethal radiation damage. *J. Food Prot.* 61:586–590.
112. Maalej, S., I. Dammak, and S. Dukan. 2006. The impairment of superoxide dismutase coordinates the derepression of the PerR regulon in the response of *Staphylococcus aureus* to HOCl stress. *Microbiology* 152:855–861.
113. Mackey, B. M. 1984. Lethal and sublethal effects of refrigeration, freezing and freeze-drying on micro-organisms, p. 45–75. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
114. Mackey, B. M. 2000. Injured bacteria, p. 315–341. *In* A. M. Lund, T. C. Baird-Parker, and G. W. Gould (ed.), The microbiological safety and quality of food. Aspen Publishers, Inc., Gaithersburg, MD.
115. Mackey, B. M., and C. M. Derrick. 1982. The effect of sublethal injury by heating, freezing, drying and gamma-radiation on the duration of the lag phase of *Salmonella typhimurium. J. Appl. Bacteriol.* 53:243–251.
116. Mackey, B. M., and C. M. Derrick. 1986. Elevation of the heat resistance of *Salmonella typhimurium* by sublethal heat shock. *J. Appl. Bacteriol.* 61:389–393.
117. Mackey, B. M., and C. M. Derrick. 1987. Changes in the heat resistance of *Salmonella typhimurium* during heating at rising temperatures. *Lett. Appl. Microbiol.* 4:13–16.
118. Mackey, B. M., and C. M. Derrick. 1987. The effect of prior heat shock on the thermoresistance of *Salmonella thompson* in foods. *Lett. Appl. Microbiol.* 5:115–118.
119. Mackey, B. M., and D. A. Seymour. 1987. The effect of catalase on recovery of heat-injured DNA-repair mutants of *Escherichia coli. J. Gen. Microbiol.* 133:1601–1610.
120. Marthi, B., B. T. Shaffer, B. Lighthart, and L. Ganio. 1991. Resuscitation effects of catalase on airborne bacteria. *Appl. Environ. Microbiol.* 57:2775–2776.
121. Martin, S. E., R. S. Flowers, and Z. J Ordal. 1976. Catalase: its effect on microbial enumeration. *Appl. Environ. Microbiol.* 32:731–734.
122. Martinez-Rodriguez, A., and B. M. Mackey. 2005. Factors affecting the pressure resistance of some *Campylobacter* species. *Lett. Appl. Microbiol.* 41:321–326.

123. Mashino, T., and I. Fridovich. 1988. Reactions of hypochlorite with catalase. *Biochem. Biophys. Acta* 956:63–69.
124. Mathew, F. P., and E. T. Ryser. 2002. Competition of thermally injured *Listeria monocytogenes* with a mesophilic lactic acid starter culture in milk for various heat treatments. *J. Food Prot.* 65:643–650.
125. Matin, A. 1991. The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. *Mol. Microbiol.* 5:3–10.
126. Mattick, K. L., F. Jorgensen, J. D. Legan, M. B. Cole, J. Porter, H. M. Lappin-Scott, and T. J. Humphrey. 2000. Survival and filamentation of *Salmonella enterica* serovar Enteritidis PT4 and *Salmonella enterica* serovar Typhimurium DT104 at low water activity. *Appl. Environ. Microbiol.* 66:1274–1279.
127. Mattick, K. L., L. E. Phillips, F. Jorgensen, H. M. Lappin-Scott, and T. J. Humphrey. 2003. Filament formation by *Salmonella* spp. inoculated into liquid food matrices at refrigeration temperatures, and growth patterns when warmed. *J. Food Prot.* 66:215–219.
128. Maurelli, A. T. 1989. Regulation of virulence genes in *Shigella*. *Mol. Biol. Med.* 6:425–432
129. Mayr, B., T. Kaplan, S. Lechner, and S. Scherer. 1996. Identification and purification of a family of dimeric major cold shock protein homologs from the psychrotrophic *Bacillus cereus* WSBC 10201. *J. Bacteriol.* 178:2916–2925.
130. Mazzotta, A. S. 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* in fruit juices. *J. Food Prot.* 64:315–320.
131. McDonald, L. C., C. R. Hackney, and B. Ray. 1983. Enhanced recovery of injured *Escherichia coli* by compounds that degrade hydrogen peroxide or block its formation. *Appl. Environ. Microbiol.* 45:360–365.
132. McGovern, V. P., and J. D. Oliver. 1995. Induction of cold responsive proteins in *Vibrio vulnificus*. *J. Bacteriol.* 177:4131–4133.
133. McMahon, M. A. S., D. A. McDowell, and I. S. Blair. 2007. The pattern of pleiomorphism in stressed *Salmonella* Virchow populations is nutrient and growth phase dependent. *Lett. Appl. Microbiol.* 45:276–281.
134. Mekalanos, J. J. 1992. Environmental signals controlling expression of virulence determinants in bacteria. *J. Bacteriol.* 174:1–7.
135. Meyer, D. H., and C. W. Donnelly. 1992. Effect of incubation temperature on repair of heat-injured *Listeria* in milk. *J. Food Prot.* 55:579–582.
136. Miller, A. J., D. O. Bayles, and B. S. Eblen. 2000. Cold shock induction of thermal sensitivity in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 66:4345–4350.
137. Mitchell, D. O., and M. J. Starzyk. 1975. Survival of *Salmonella* and other indicator microorganisms. *Can. J. Microbiol.* 21:1420–1421.
138. Mossel, D. A. A., and P. van Netten. 1984. Harmful effects of selective media on stressed micro-organisms: nature and remedies, p. 329–369. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
139. Mukamolova, G. V., A. S. Kaprelyants, D. I. Young, M. Young, and D. B. Kell. 1998. A bacterial cytokine. *Proc. Natl. Acad. Sci. USA* 95:8916–8921.
140. Murakami, K., T. Ono, D. Viducic, S. Kayama, M. Mori, K. Hirota, K. Nemoto, and Y. Miyake. 2005. Role for rpoS gene of *Pseudomonas aeruginosa* is antibiotic tolerance. *FEMS Microbiol. Lett.* 242:161–167.
141. Murano, E. A., and M. D. Pierson. 1992. Effect of heat shock and growth atmosphere on the heat resistance of *Escherichia coli* O157:H7. *J. Food Prot.* 55:171–175.
142. Murano, E. A., and M. D. Pierson. 1993. Effect of heat shock and incubation atmosphere on injury and recovery of *Escherichia coli* O157:H7. *J. Food Prot.* 56:568–572.
143. Musarrat, J., and M. Ahmad. 1988. pH induced damage and repair in *E. coli*. *Mutat. Res.* 193:219–227.
144. Neidhardt, F. C., and R. A. VanBogelen. 1987. Heat shock response, p. 1334–1345. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella Typhimurium*: cellular and molecular biology. ASM Press, Washington, DC.
145. Novak, J. S., M. H. Tunick, and V. K. Juneja. 2001. Heat treatment adaptations in *Clostridium perfringens* vegetative cells. *J. Food Prot.* 64:1527–1534.
146. O'Driscoll, B., C. G. M. Gahan, and C. Hill. 1996. Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. *Appl. Environ. Microbiol.* 62:1693–1698.
147. Oliver, J. D. 1981. Lethal cold stress of *Vibrio vulnificus* in oysters. *Appl. Environ. Microbiol.* 41:710–717.
148. Pagán, R., S. Condon, and F. J. Sala. 1997. Effects of several factors on the heat-shock-induced thermotolerance of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 63:3225–3232.
149. Pagán, R., P. Mañas, A. Palop, and F. J. Sala. 1999. Resistance of heat-shocked cells of *Listeria monocytogenes* to mano-sonication and mano-thermo-sonication. *Lett. Appl. Microbiol.* 28:71–75.
150. Palumbo, S. A. 1984. Heat injury and repair in *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 48:477–480.
151. Panutdaporn, N., K. Kawamoto, H. Asakura, and S.-I. Makino. 2006. Resuscitation of the viable but non-culturable state of *Salmonella enterica* serovar Oranienburg by recombinant resuscitation-promoting factor derived from *Salmonella* Typhimurium strain LT2. *Int. J. Food Microbiol.* 106:241–247.
152. Park, S., and J. A. Imlay. 2003. High levels of intracellular cysteine promote oxidative DNA damage by driving the Fenton reaction. *J. Bacteriol.* 185:1942–1950.
153. Pellon, J. R., and A. J. Sinskey. 1984. Heat-induced damage to the bacterial chromosome and its repair, p. 106–125. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
154. Pommepuy, M., M. B. Utin, A. Derrien, M. Gourmelon, R. R. Colwell, and M. Cormier. 1996. Retention of enteropathogenicity by a viable but non-culturable *Escherichia coli* exposed to seawater and sunlight. *Appl. Environ. Microbiol.* 62:4621–4626.
155. Pomposiello, M. H., M. H. Bennik, and B. Bennik. 2001. Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. *J. Bacteriol.* 183:3890–3902.
156. Popoff, M. Y. 2001. Antigenic formulas of the *Salmonella* serovars, 8th ed. WHO Collaborating Centre for Reference and Research on *Salmonella.* World Health Organization, Geneva.
157. Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling. 2003. Supplement 2001 (no. 45) to the Kauffmann-White scheme. *Res. Microbiol.* 154:173–174.
158. Postgate, J. R., and J. R. Hunter. 1963. The survival of starved bacteria. *J. Appl. Bacteriol.* 26:295–306.
159. Prentice, G. A., and L. F. I. Clegg. 1974. The effects of incubation temperature on the recovery of spores of *Bacillus subtilis* 2057. *J. Appl. Bacteriol.* 37:501–508.
160. Przybylski, K. S., and L. D. Witter. 1979. Injury and recovery of *Escherichia coli* after sublethal acidification. *Appl. Environ. Microbiol.* 37:261–265.
161. Purvis, J. E., L. P. Yomano, and L. O. Ingram. 2005. Enhanced trehalose production improves growth of *Escherichia coli* under osmotic stress. *Appl. Environ. Microbiol.* 71:3761–3769.
162. Quesnel, L. B. 1984. Biological indicators and sterilization processes, p. 257–291. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
163. Quintavalla, S., and M. Campanini. 1991. Effect of rising temperature on the heat resistance of *Listeria monocytogenes* in meat emulsion. *Lett. Appl. Microbiol.* 12:184–187.
164. Rahman, I., M. Shahamat, P. A. Kirchman, E. Russek-Cohen, and R. R. Colwell. 1994. Methionine uptake and cytopathogenicity of viable but nonculturable *Shigella dysenteriae* type 1. *Appl. Environ. Microbiol.* 60:3573–3578.
165. Ray, B. 1984. Reversible freeze-injury, p. 237–271. *In* A. Hurst and A. Nasim (ed.), Repairable lesions in microorganisms. Academic Press, London.

166. Ray, B. 1986. Impact of bacterial injury and repair in food microbiology: its past, present and future. *J. Food Prot.* 49:651–655.
167. Ray, B. 1989. Injured index and pathogenic bacteria: occurrence and detection in foods, water and feeds. CRC Press, Boca Raton, FL.
168. Ray, B. 2001. Fundamental food microbiology, 2nd ed. CRC Press, Boca Raton, FL.
169. Rees, C. E. D., C. E. R. Dodd, P. T. Gibson, L. R. Booth, and G. S. A. B. Stewart. 1995. The significance of bacteria in stationary phase to food microbiology. *Int. J. Food Microbiol.* 28:263–275.
170. Restaino, L., W. S. Jeter, and W. M. Hill. 1980. Thermal injury of *Yersinia enterocolitica*. *Appl. Environ. Microbiol.* 40:939–949.
171. Robinson, T. P., M. J. Ocio, A. Kaloti, and B. M. Mackey. 1998. The effect of growth environment on the lag phase of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 44:83–92.
172. Rosen, H., and S. J. Klebanoff. 1982. Oxidation of *Escherichia coli* iron centers by the myeloperoxidase-mediated microbicidal system. *J. Biol. Chem.* 257:13731–13735.
173. Rowbury, R. J. 2003. Physiology and molecular basis of stress adaptation, with particular reference to the subversion of stress adaptation, and to the involvement of extracellular components in adaptation, p. 247–302. *In* A. E. Yousef and V. J. Juneja (ed.), Microbial stress adaptation and food safety. CRC Press, Boca Raton, FL.
174. Rowe, M. T., and R. B. Kirk. 2000. Effect of nutrient starvation on the resistance of *Escherichia coli* O157:H7 to subsequent heat stress. *J. Food Prot.* 63:1745–1748.
175. Russell, A. D. 1984. Potential sites of damage in microorganisms exposed to chemical or physical agents, p. 1–18. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
176. Rychlik, I., and P. A. Barrow. 2005. *Salmonella* stress management and its relevance to behaviour during intestinal colonization and infection. *FEMS Microbiol. Rev.* 29:1021–1040.
177. Sampathkumar, B., G. G. Khachatourians, and D. R. Korber. 2003. High pH during trisodium phosphate treatment causes membrane damage and destruction of *Salmonella enterica* serovar Enteritidis. *Appl. Environ. Microbiol.* 69:122–129.
178. Sato, M., and H. Takahashi. 1970. Cold-shock of bacteria. IV. Involvement of DNA-ligase reaction in recovery of *Escherichia coli* from cold shock. *J. Gen. Appl. Microbiol.* 16:279–290.
179. Semanchek, J. J., and D. A. Golden. 1998. Influence of growth temperature on inactivation and injury of *Escherichia coli* O157:H7 by heat, acid, and freezing. *J. Food Prot.* 61:395–401.
180. Sharma, M., B. B. Adler, M. D. Harrison, and L. R. Beuchat. 2005. Thermal tolerance of acid-adapted and unadapted *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. *Lett. Appl. Microbiol.* 41:448–453.
181. Singh, A., and G. A. McFeters. 1987. Survival and virulence of copper- and chlorine-stressed *Yersinia enterocolitica* in experimentally infected mice. *Appl. Environ. Microbiol.* 53:1768–1774.
182. Skurnik, M., and P. Toivanen. 1992. LcrF is the temperature-regulated activator of the *yadA* gene of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *J. Bacteriol.* 174:2047–2051.
183. Sleator, R. D., and C. Hill. 2002. Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. *FEMS Microbiol. Rev.* 26:49–71.
184. Smith, J. L., and S. A. Palumbo. 1978. Injury to *Staphylococcus aureus* during sausage fermentation. *Appl. Environ. Microbiol.* 36: 857–860.
185. Snyder, L., and W. Champness. 1997. Molecular genetics of bacteria. ASM Press, Washington, DC.
186. Sokolovic, Z., A. Fuchs, and W. Goebel. 1990. Synthesis of species-specific stress proteins by virulent strains of *Listeria monocytogenes*. *Infect. Immun.* 58:3582–3587.
187. Sokolovic, Z., J. Riedel, M. Wuenscher, and W. Goebel. 1993. Surface-associated, PrfA-regulated proteins of *Listeria monocytogenes* synthesized under stress conditions. *Mol. Microbiol.* 8:219–227.
188. Stapleton, G. E., D. Billen, and A. Hollaender. 1953. Recovery from x-irradiated bacteria at suboptimal incubation temperatures. *J. Cell Comp. Physiol.* 41:345–358.
189. Stephens, P. J., M. B. Cole, and M. V. Jones. 1994. Effect of heating rate on the thermal inactivation of *Listeria monocytogenes*. *J. Appl. Bacteriol.* 77:702–708.
190. Stephens, P. J., P. Druggan, and G. Nebe-von-Caron. 2000. Stressed *Salmonella* are exposed to reactive oxygen species from two independent sources during recovery in conventional culture media. *Int. J. Food Microbiol.* 60:269–285.
191. Stephens, P. J., J. A. Joynson, K. W. Davies, R. Holbrook, H. M. Lappin-Scott, and T. J. Humphrey. 1997. The use of an automated growth analyzer to measure recovery times of single heat-injured *Salmonella* cells. *J. Appl. Microbiol.* 83:445–455.
192. Stern, N. J., D. M. Jones, I. V. Wesley, and D. M. Rollins. 1994. Colonization of chicks by nonculturable *Campylobacter* spp. *Lett. Appl. Microbiol.* 18:333–336.
193. Storz, G., and R. Hengge-Aronis. 2000. Preface, p. xii–xiv. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, DC.
194. Sunil, B. 2000. The true impact of food borne infections. *J. Indian Vet. Assoc. Online.* Available at: http://www.jivaonline.com. Accessed 8 May 2008.
195. Sykes, G. 1963. The phenomenon of bacterial survival. *J. Appl. Bacteriol.* 26:287–294.
196. Taormina, P. J., and L. R. Beuchat. 2001. Survival and heat resistance of *Listeria monocytogenes* after exposure to alkali and chlorine. *Appl. Environ. Microbiol.* 67:2555–2563.
197. Thanomsub, B., V. Anupunpisit, S. Chanphetch, T. Watcharachaipong, R. Poonkhum, and C. Srisukonth. 2002. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. *J. Gen. Appl. Microbiol.* 48:193–199.
198. Tolker-Nielsen, T., and S. Molin. 1996. Role of ribosome degradation in the death of heat-stressed *Salmonella typhimurium*. *FEMS Microbiol. Lett.* 142:155–160.
199. Touati, D. 2000. Iron and oxidative stress in bacteria. *Arch. Biochem. Biophys.* 373:1–6.
200. Touati, D., M. Jacques, B. Tardat, L. Bouchard, and S. Despied. 1995. Lethal oxidative damage and mutagenesis are generated by iron in Δ*fur* mutants of *Escherichia coli*: protective role of superoxide dismutase. *J. Bacteriol.* 177:2305–2314.
201. Tsuchido, T., R. A. VanBogelen, and F. C. Neidhardt. 1986. Heat shock response in *Escherichia coli* influences cell division. *Proc. Natl. Acad. Sci. USA* 83:6959–6963.
202. Valentine, J. S., C. S. Foote, A. Greenberg, and J. L. Liebmen. 1995. Active oxygen in biochemistry. Blackie, London. As quoted in P. J. Stephens, P. Druggan, and G. Nebe-von-Caron. 2000. Stressed *Salmonella* are exposed to reactive oxygen species from two independent sources during recovery in conventional culture media. *Int. J. Food Microbiol.* 60:269–285.
203. Valone, S. E., G. K. Chikami, and V. L. Miller. 1993. Stress induction of the virulence proteins (SpvA, -B, and -C) from native plasmid pSDL2 of *Salmonella dublin*. *Infect. Immun.* 61:705–713.
204. VanBogelen, R. A., and F. C. Neidhardt. 1990. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 87:5589–5593.
205. Van Schothorst, M., and A. M. Duke. 1984. Effect of sample handling on microbial limits laid down in standards, p. 309–328. *In* M. H. E. Andrew and A. D. Russell (ed.), The revival of injured microbes. Academic Press, London.
206. Virto, R., P. Mañas, I. Álvarez, S. Condon, and J. Raso. 2005. Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Appl. Environ. Microbiol.* 71:5022–5028.
207. Völker, U., H. Mach, R. Schmid, and M. Hecker. 1992. Stress proteins and cross-protection by heat shock and salt stress in *Bacillus subtilis*. *J. Gen. Microbiol.* 138:2125–2135.
208. Watson, K. 1990. Microbial stress proteins. *Adv. Microbiol. Physiol.* 31:183–223.
209. Wemekamp-Kamphuis, H. H., J. A. Wouters, P. P. L. A. de Leeuw, T. Hain, T. Chakraborty, and T. Abee. 2004. Identification of sigma

factor σ^B-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. *Appl. Environ. Microbiol.* 70:3457–3466.

210. Wesche, A. M., B. P. Marks, and E. T. Ryser. 2005. Thermal resistance of heat-, cold-, and starve-injured *Salmonella* in irradiated comminuted turkey. *J. Food Prot.* 68:942–948.
211. Wong, H.-C., P.-Y. Peng, S.-L. Lan, Y.-C. Chen, K.-H. Lu, C.-T. Shen, and S.-F. Lan. 2002. Effects of heat shock on the thermotolerance, protein composition, and toxin production of *Vibrio parahaemolyticus*. *J. Food Prot.* 65:499–507.
212. Wouters, J. A., F. M. Rombouts, O. P. Kuipers, W. M. de Vos, and T. Abee. 2000. The role of cold-shock proteins in low-temperature adaptation of food-related bacteria. *Syst. Appl. Microbiol.* 23:165–173.
213. Wouters, J. A., J.-W. Sanders, J. Kok, W. M. De Vos, O. P. Kuipers, and T. Abee. 1998. Clustered organization and transcriptional analysis of a family of five *csp* genes of *Lactococcus lactis* MG1363. *Microbiology* 144:2885–2893.
214. Wright, C. T., and T. R. Klaenhammer. 1981. Calcium-induced alterations in cellular morphology affecting the resistance of *Lactobacillus acidophilus* to freezing. *Appl. Environ. Microbiol.* 41:807–815.
215. Wuytack, E. Y., L. D. T. Phuong, A. Aertsen, K. M. F. Reyns, D. Marquenie, B. De Ketelaere, B. Masschalck, I. Van Opstal, A. M. J. Diels, and C. W. Michiels. 2003. Comparison of sublethal injury induced in *Salmonella enterica* serovar Typhimurium by heat and by different nonthermal treatments. *J. Food Prot.* 66:31–37.
216. Yamamoto, T., T. Hanawa, and S. Ogata. 1994. Induction of *Yersinia enterocolitica* stress proteins by phagocytosis with macrophage. *Microbiol. Immunol.* 38:295–300.
217. Yan, Z., J. B. Gurtler, and J. L. Kornacki. 2006. A solid agar overlay method for recovery of heat-injured *Listeria monocytogenes*. *J. Food Prot.* 69:428–431
218. Yousef, A. E., and P. D. Courtney. 2003. Basics of stress adaptation and implications in new-generation foods, p. 1–30. *In* A. E. Yousef and V. J. Juneja (ed.), Microbial stress adaptation and food safety. CRC Press, Boca Raton, FL.
219. Yuk, H.-G., and D. L. Marshall. 2004. Adaptation of *Escherichia coli* O157:H7 to pH alters membrane lipid composition, verotoxin secretion, and resistance to simulated gastric fluid. *Appl. Environ. Microbiol.* 70:3500–3505.
220. Yuk, H.-G., and D. L. Marshall. 2005. Influence of acetic, citric, and lactic acids on *Escherichia coli* O157:H7 membrane lipid composition, verotoxin secretion, and acid resistance in simulated gastric fluid. *J. Food Prot.* 68:673–679.
221. Yuk, H.-G., and K. R. Schneider. 2006. Adaptation of *Salmonella* spp. in juice stored under refrigerated and room temperature enhances resistance to simulated gastric fluid. *Food Microbiol.* 23: 694–700.
222. Zhao, T., and M. P. Doyle. 2001. Evaluation of universal preenrichment broth for growth of heat-injured pathogens. *J. Food Prot.* 64:1751–1755.
223. Zheung, M., X. Wang, L. J. Templeton, D. R. Smulski, R. A. LaRossa, and G. Storz. 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* 183:4562–4570.
224. Zook, C. D., F. F. Busta, and L. J. Brady. 2001. Sublethal sanitizer stress and adaptive response of *E. coli* O157:H7. *J. Food Prot.* 64: 767–769.
225. Zugel, U., and S. H. Kaufmann. 1999. Role of heat shock proteins in protection from pathogenesis of infectious diseases. *Clin. Microbiol. Rev.* 12:19–39.